




Xylem vessel-diameter–shoot-length scaling: ecological significance of porosity types and other traits

MARK OLSON ^{1,7}, JULIETA A. ROSELL ², CECILIA MARTÍNEZ-PÉREZ,¹ CALIXTO LEÓN-GÓMEZ,¹ ALEX FAJARDO ³,
 SANDRINE ISNARD,^{4,5} MARÍA ANGÉLICA CERVANTES-ALCAYDE,¹ ALBERTO ECHEVERRÍA,¹ VÍCTOR A. FIGUEROA-ABUNDIZ,¹
 ALÍ SEGOVIA-RIVAS,¹ SANTIAGO TRUEBA,^{4,5,6} AND KAREN VÁZQUEZ-SEGOVIA²

¹*Instituto de Biología, Universidad Nacional Autónoma de México, Tercer Circuito s/n de Ciudad Universitaria, Ciudad de México 04510 México*

²*Laboratorio Nacional de Ciencias de la Sostenibilidad, Instituto de Ecología, Universidad Nacional Autónoma de México, Tercer Circuito s/n de Ciudad Universitaria, Ciudad de México 04510 México*

³*Centro de Investigación en Ecosistemas de la Patagonia (CIEP), Camino Baguales s/n, Coyhaique 5951601 Chile*

⁴*Botanique et Modélisation de l'Architecture de Plantes de des Végétations, Institut de Recherche pour le Développement, Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Centre National de la Recherche Scientifique, Institut National de la Recherche Agronomique, Université de Montpellier, Montpellier, 34398, France*

⁵*Botanique et Modélisation de l'Architecture de Plantes de des Végétations, Institut de Recherche pour le Développement, Herbier de Nouvelle-Calédonie, Nouméa, 98848, New Caledonia*

⁶*School of Forestry & Environmental Studies, Yale University, New Haven, Connecticut 06511 USA*

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Abstract. Flowering plants predominantly conduct water in tubes known as vessels, with vessel diameter playing a crucial role in plant adaptation to climate and reactions to climate change. The importance of vessels makes it essential to understand how and why vessel diameter, plant height, and other ecological factors are interrelated. Although shoot length is by far the main driver of variation in mean vessel diameter across angiosperms, much remains to be understood regarding the factors accounting for the abundant variation around the y-axis in plots of mean species vessel diameter against shoot length. Here, we explore the potential role of porosity types, wood density, leaf phenology, background imperforate tracheary element type, vasicentric tracheids, vascular tracheids, perforation plate type, and successive cambia in causing variation in the y-intercept or slope of the mean species vessel-diameter– and vessel-density–shoot-length associations at the shoot tip and base. We detected numerous cases of ecologically significant variation. For example, latewood vessels of ring porous species scale with a lower slope than earlywood, i.e., latewood vessels are relatively narrow in taller plants. This pattern is likely the result of selection favoring freezing-induced embolism resistance via narrow vessels. Wood density was negatively associated with vessel diameter, with low wood density plants having wider vessels for a given height. Species with scalariform perforation plates scale with a lower shoot base vessel-diameter–shoot-length slope, likely reflecting selection against scalariform plates in wide vessels. In other cases, functional groups scaled similarly. For example, species with successive cambia did not differ from those with conventional single cambia in their mean vessel-diameter–shoot-length scaling, rejecting our prediction that species with successive cambia should have narrower vessels for a given shoot length. They did, however, have fewer vessels per unit shoot cross-sectional area than plants of similar heights, likely because vessels have longer functional lifespans (and therefore are fewer) in species with successive cambia. Our methods illustrate how vessel diameter can be studied taking shoot length into account to detect ecologically important variation and construct theory regarding plant adaptation via the hydraulic system that includes plant size as a vital element.

Key words: allometry; leaf phenology; porosity type; scalariform perforation plate; wood density; xylem vessel.

INTRODUCTION

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⁷ E-mail: molson@ib.unam.mx

Across the flowering plants, a crucial nexus between photosynthetic productivity, climate, soil, and habit is

the hydraulic system. The hydraulic system is the locus of transport of water between the sites of uptake in the roots and the sites of liberation to the atmosphere in the leaves, and in flowering plants mostly takes place in conduits known as vessels. Across the angiosperms, vessels share the characteristics of being made up of individual cells called vessel elements (tubular cells, dead at maturity) joined end to end to form conduits of varying lengths, with water flowing from element to element through holes known as perforations. Overlain on these common features is myriad variation in the characteristics of the vessels themselves, the way that vessels are deployed throughout shoots, the kinds of cells that surround them, as well as variation in plant habit and leaf phenology. The conductive system is a key participant in habit diversification of angiosperms (Carlquist 1980, 2001, Rosell and Olson 2014, Isnard and Feild 2015) and in the diversification of these plants into different climates (Zanne et al. 2018). Given this importance, it is essential to understand the ecological significance of variation in vessel characteristics across the flowering plants.

Such an understanding has proven challenging because of the confounding effects of plant height with the effects of climatic, structural, phenological, and habit variation on vessel characteristics. Traditionally, the effects of factors such as climate or habit are studied as though conduit characteristics were a direct reflection of ecological contexts independently of plant height (Apgaua et al. 2015, Warwick et al. 2017, Zanne et al. 2018). From an operational point of view, this means that correlations between variation in vessel characteristics with factors such as climate, habit, or other factors are studied directly, without taking plant height into account. However, across the vessel-bearing angiosperms, vessel diameter scales predictably with plant height or shoot length (or, because of generally predictable length–diameter allometry, with shoot diameter), regardless of phylogenetic affinity, plant habit, or habitat (Fig. 1); for self-supporting plants, shoot length, that is, distance from the trunk base to the tip of the distalmost twig, and height (we use the term “shoot length” throughout) should be similar and closely reflect conductive path length (Anfodillo et al. 2006, McCulloh et al. 2010, Olson et al. 2013, 2014, Rosell and Olson 2014, Lechthaler et al. 2018, Olson et al. 2018b). This means that finding that one individual has wider vessels than another might simply be a reflection of the longer shoot length of that individual, with the two individuals sampled from along the same vessel-diameter–shoot-length scaling trajectory. Existing statistical techniques, such as ANCOVAs, are available for testing the effect of environment or other variables on vessel diameter while taking shoot length into account. Height standardization has yet to be widely applied, but results to date suggest that the long-recognized relationship between conduit diameter and climate requires modification. Mean species vessel diameter is traditionally regarded as

being narrow in cold, dry places, independently of shoot length. This pattern is traditionally explained as the result of selection favoring narrow vessels in dry and cold situations because of their greater resistance to freezing- and probably drought-induced embolism (Langan et al. 1997, Davis et al. 1999, Cavender-Bares and Holbrook 2001, Martínez-Vilalta and Pockman 2002, Pittermann and Sperry 2003, Cavender-Bares 2005, Cavender-Bares et al. 2005, Stuart et al. 2007, Cai and Tyree 2010, Savage and Cavender-Bares 2013, Zanne et al. 2013, Jacobsen et al. 2019, Liu et al. 2019). However, recent work shows that vessel-diameter–climate associations largely disappear when plant height is taken into account. For example, without taking shoot length into account, mean vessel diameter varies markedly with precipitation, with significantly wider vessel diameters in moister localities (Carlquist and Hoekman 1985, Warwick et al. 2017). Taking shoot length into account, though, shows that plants across habitats differing in water availability have similar vessel-diameter–shoot-length y -intercepts (Fajardo et al. 2020). That is, plants growing in areas of higher precipitation do not have wider vessels for a given height than those in dry areas (Olson et al. 2014, Morris et al. 2018, Olson et al. 2018b). The tendency for mean vessel diameter to be wider in moister areas is due to the taller maximum plant heights, height variances, mean plant heights, and therefore community mean vessel diameters, in moister areas. Therefore, it would be useful to revisit many ecological patterns of association involving conduit diameter in the light of conduit scaling with shoot length (Olson et al. 2013, 2014, Rosell et al. 2017a, Lechthaler et al. 2018, Morris et al. 2018, Olson et al. 2018b, Rodriguez-Zaccaro et al. 2019).

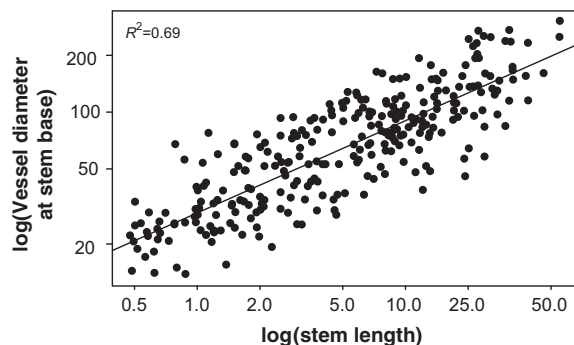


FIG. 1. Vessel diameter (measured in μm) scales predictably with shoot length (measured in m). Each point represents a species-level mean value, with data from 283 species, including trees, shrubs, lianas, succulents, and other plant life forms. The scaling slope coincides with the rate predicted based on optimality models to minimize drops in per-leaf area conductance with height growth. While shoot length is clearly the main driver of mean species vessel diameter, the variation about the scaling slope indicates that there is a range of vessel diameter that can be observed for a given shoot length. In the present paper, we investigate several major potential causes of this variation. Data from Rosell and Olson (2014).

There are compelling reasons to expect plant conduit diameter to scale with plant size. Scaling of conduit diameter with shoot length is expected as the result of natural selection favoring the maintenance of constant conductance per unit leaf area as individual plants grow taller and their conductive path lengths become longer (West et al. 1999, Enquist 2003, Olson et al. 2018b). The longer path lengths that accompany longer shoots are a significant selection pressure because, assuming constant tissue-level properties, hydraulic resistance increases linearly with increases in conductive path length (Vogel 2003). Given heritable variation between the individuals within a species, those with conduits that remain “too narrow” are expected to experience increasing resistance with height growth, lowered conductance, continually lowered photosynthetic productivity, and continually less surplus carbon to devote to growth and reproduction. Those with conduits that become “too wide” as they grow taller will have excessively wide conduits of higher per-conduit carbon cost. Moreover, their potentially higher embolism vulnerability will lead to lowered conductance, lowered photosynthetic productivity, and continually less surplus carbon to devote to growth and reproduction. Variants whose vessels widen just enough such that they maintain leaf-specific conductance constant with height growth (Enquist 2003, Selaya and Anten 2010, Michaletz et al. 2014, Stephenson et al. 2014, Echeverría et al. 2019; see also Roddy et al. 2019), but with conduits not widening so much that carbon costs are excessive and embolism risk too high, should have maximal surplus carbon to devote to growth and reproduction. Selection therefore appears to favor the mean diameter of conduits increasing predictably from the tip down branches and the trunk of the tree, across the vascular plants. The empirical rate of this widening is very close to that predicted in models of plant hydraulic function to maintain conductance constant as plants grow taller (West et al. 1997, 1999, Anfodillo et al. 2006, Petit and Anfodillo 2009, Olson and Arroyo-Santos 2015, Olson et al. 2018b, Williams et al. 2019). Because the factors mentioned above (such as vessel anatomical characteristics, the deployment of vessels in shoots, the cells surrounding vessels, and habit and leaf phenology) can potentially cause ecologically significant variation in vessel diameter (McCulloh et al. 2010, Rosell et al. 2017a, Medeiros et al. 2018, Liu et al. 2019), it is essential to understand how the general pattern of vessel scaling with height is intertwined with ecologically important variation in vessel diameter.

Using a comparative study across the woody vessel-bearing “dicots” (angiosperms minus the monocots, in quotes because the group is paraphyletic), we addressed whether major differences across species in porosity type (ring, semi-ring, and diffuse porous), wood density, leaf phenology (drought deciduous, evergreen, and cold deciduous), imperforate tracheary element type (tracheids, fiber tracheids, libriform fibers, and vasicentric and vascular tracheids), perforation plate type (scalariform

and simple), and successive cambia cause variation in the y -intercept or slope of the mean species vessel diameter and vessel density associations with shoot length at the shoot tip and base. We now turn to each of these considerations in more detail, as well as sketching our ecological predictions and the general statistical rationale used to test them.

The many types of growth rings in woods (Carlquist 2001) can be classed into three major categories known as porosity types (ring porous, semi-ring porous, and diffuse porous, defined in Table 1 and illustrated in Fig. 2a–c), and there are some reasons to expect differences in the vessel-diameter–shoot-length relationship across these categories. Ring porosity is characterized by very wide vessels in the first-formed secondary xylem of the growing season (earlywood), followed by markedly narrow vessels at the end of the growing season (latewood). Ring porous species have been found to have steeper conduit-diameter–shoot-diameter slopes than other species (McCulloh et al. 2010), and this might indicate differences in vessel-diameter–height scaling. Ring porosity is nearly restricted to the north temperate zone and is relatively uncommon at a global level (Wheeler et al. 2007, McCulloh et al. 2010). In contrast, diffuse porosity is very widespread, found worldwide and in practically all habitats. Diffuse porous plants have no marked variation in vessel diameter across seasons. As a result, in contrast to ring porous species, in diffuse porous species, vessels of wide and narrow size classes are produced throughout the year, and because they experience the same environment, would not be expected to differ in their scaling relationships with height. By contrast, semi-ring porous species have vessels that alter in diameter or density from earlywood to latewood, but gradually rather than abruptly as in ring porous species. These often occur in cold-prone and Mediterranean areas (Wheeler et al. 2007), where selection could potentially favor relatively narrow vessels for a given shoot length. There are thus reasons to expect that porosity types could affect vessel-diameter–shoot-length scaling.

With regard to our general approach for testing for differences associated with the traits mentioned above, the main driver of variation in vessel diameter is by far shoot length, so testing the relationship between porosity type or other anatomical features needs to be carried out in the context of the vessel-diameter–shoot-length relationship. That is, porosity or other features can potentially explain the residual variation in vessel-diameter–shoot-length scaling. Ring porous species might have higher or lower vessel-diameter–shoot-length scaling slopes than other plants, or they might have higher or lower intercepts. Higher or lower intercepts imply wider or narrower vessels for a given shoot length, which would in turn suggest differences in hydraulic behavior between individuals. We used analogous reasoning to examine several other important variables for their effects on vessel-diameter–shoot-length scaling, and we summarize these briefly.

TABLE 1. Delimitations of the states of the categorical characters studied.

Character and states	Definitions
Porosity types	
Ring porous	Very wide earlywood vessels that transition rapidly to narrow latewood vessels or even vascular tracheids (Carlquist 1985, Rosell et al. 2007).
Semi-ring porous	Vessels diminish in diameter across growth rings gradually (Carlquist 2001, Medeiros and Pockman 2014).
Diffuse porous	Growth rings, if present, can be diagnosed by differences in imperforate tracheary element diameter, parenchyma bands, or other features, but vessels do not vary markedly across growth rings in their diameter or abundance, or growth rings absent.
Leaf phenology	
Evergreen	Leaves are retained all year round.
Cold deciduous	Leaves are dropped during a freezing-prone portion of the year, often moist habitats.
Drought deciduous	Leaves are dropped during a dry portion of the year, often frost-free habitats.
Leafless	Non-succulent plants that lack leaves, e.g., <i>Casuarina</i> , <i>Jacksonia</i> . Excluded from phenology analysis because of low frequency in data set.
Stem succulent	Often leafless or largely leafless plants that perform most of their photosynthesis in thick, water-storing stems, e.g., <i>Pachycereus</i> , <i>Euphorbia cyri</i> . Excluded from phenology analysis because of low frequency in data set.
Perforation plate type	
Scalariform	All or most vessel elements have perforation plates traversed by anywhere from 10 to dozens of parallel bars, the plate thus resembling a ladder.
Simple	Perforation plates lack bars.
Imperforate tracheary elements	
True tracheids	Have abundant pits with distinct borders, similar in size and abundance to those on vessel-vessel walls. Thought to be conductive.
Fiber tracheids	Have pits with distinct borders, which are either similar in size but less abundant than those of lateral vessel walls, or have borders that are narrower than vessel-vessel pits. Thought to be non-conductive.
Libriform fibers	Have pits that lack borders. Thought to be non-conductive; often living.
Vasicentric tracheids	Conductive imperforate tracheary elements present in addition to libriform fibers or fiber tracheids; in most species, they resemble narrow vessel elements lacking perforation plates (e.g., not elongating beyond the length of the fusiform cambial initials they are derived from). In some species with fiber tracheids, they resemble true tracheids (e.g., in undergoing elongation beyond the lengths of the fusiform cambial initials). Vasicentric tracheids are always present adjacent to vessels and are thought to provide an alternate conductive stream that is more resistant to embolism than vessels.
Vascular tracheids	Similar to vasicentric tracheids but present to the exclusion of vessels in latewood. They can be found in species with or without vasicentric tracheids.
Vascular cambium (produces secondary phloem to the outside and secondary xylem to the inside)	
Single conventional	In larger shoots, a single vascular cambium is continuous around the shoot, with no xylem produced external to the phloem. The secondary phloem forms the bulk of the bark (Rosell et al. 2017b). As the shoot widens, the distance between a given vessel and living phloem increases.
Successive	Multiple vascular cambia are produced, in concentric bands or as strands, whose products are separated by thin-walled or sclerified conjunctive tissue. The bark lacks phloem. Vessels remain in proximity to living phloem.

Leaf phenology, understood as the distinction between deciduous and evergreen species, is also often cited as being potentially associated with hydraulic differences (Wang et al. 1992, Cavender-Bares and Holbrook 2001, Cavender-Bares et al. 2005, Chen et al. 2009, Méndez-Alonzo et al. 2013, Savage and Cavender-Bares 2013, Wolfe et al. 2016). This is because whether leaves are shed or persist into drought or cold should impose varying challenges with regard to embolism formation. These differences could be reflected in differences in the slopes or

intercepts of the vessel-diameter–shoot-length relationship across phenological categories we examined (evergreen, drought deciduous, and cold deciduous; see Table 1).

A major determinant of hydraulic performance is perforation plate type, with the fundamental distinction being scalariform vs. simple plates. Carlquist (2018) has recently proposed the hypothesis that, because they add friction to the flow of the water column (Christman and Sperry 2010), scalariform perforation plates should only be retained in situations of high resistance, such as in

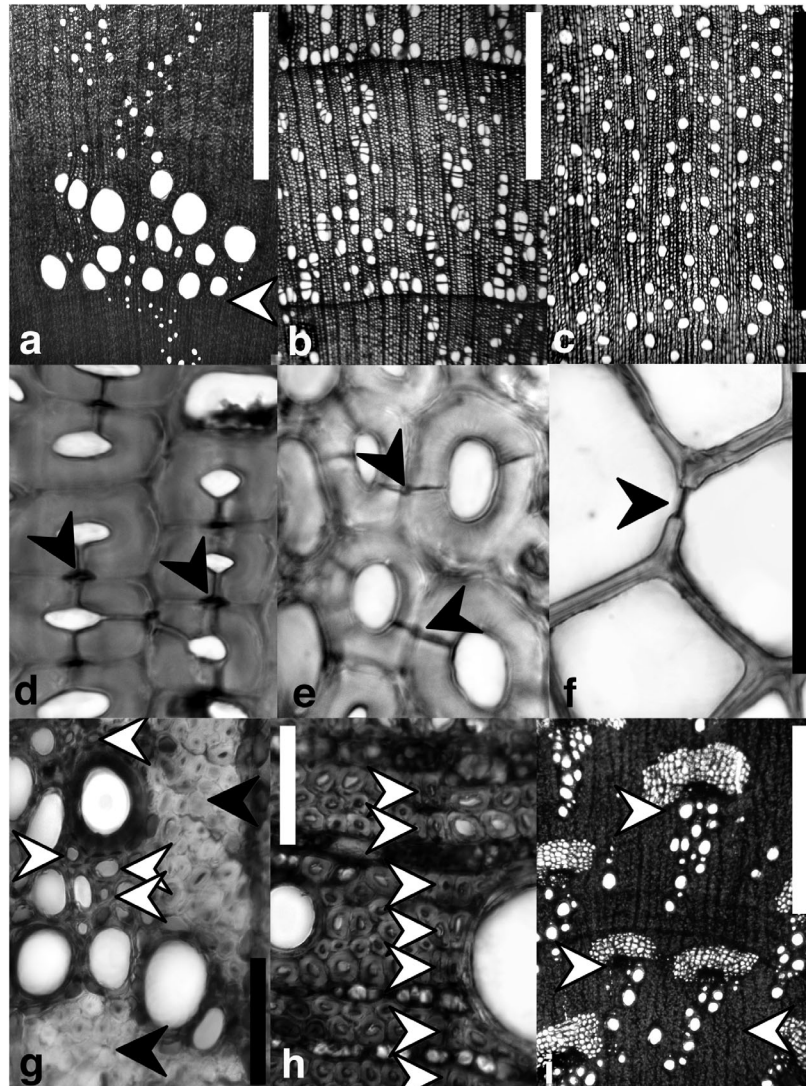


FIG. 2. Photomicrographs of transections showing porosity types, imperforate tracheary element types, and successive cambia. Unless noted, images are oriented with the cambium at top. (a–c) Porosity types. (a) Ring porosity is characterized by very wide vessels at the beginning of a growth ring (white arrow marks the end of the previous ring) followed by an abrupt transition to narrow vessels. *Quercus lobata* (Fagaceae, Fagales). (b) Semi-ring porosity is characterized by a gradual decrease in vessel diameter or abundance, or both, as in this *Corylus avellana* (Betulaceae, Fagales). (c) In diffuse porous species, as in *Polylepis rugulosa* (Rosaceae, Rosales), the vessels do not change in diameter or abundance. (d–f) Background imperforate tracheary elements. (d) True tracheids have abundant pits with conspicuous pit chambers (“borders”; arrows) and are thought to be conductive. *Quintinia major* (Paracryphiaceae, Paracryphiales). (e) Fiber tracheids are often pitted more sparsely than true tracheids, and the pit chambers are small (arrows). Fiber tracheids are thought to be non-conductive. *Randia* sp. (Rubiaceae, Gentianales). (f) The pits of libriform fibers lack chambers entirely (arrow). *Moringa oleifera* (Moringaceae, Brassicales). (g) Some species with libriform fibers (as in this case; black arrow) or fiber tracheids have, in addition, vasicentric tracheids, conductive imperforate tracheary elements that surround vessels. With their easily seen pits with distinct pit chambers, vasicentric tracheids (white arrows) contrast conspicuously with libriform fibers. *Ottleya rigida* (Fabaceae, Fabales). (h) Vascular tracheids are a latewood band of conductive imperforate tracheary elements in species with fiber tracheids or, as in this *Adelia brandegeei* (Euphorbiaceae, Malpighiales), libriform fibers. The cambium is at right, with a latewood band two cells thick of vascular tracheids (arrows) terminating the latewood. The vascular tracheids have conspicuous pit chambers, in contrast to the libriform fibers that surround them. The wide vessel at right is in the earlywood of the next growth ring. (i) The vessels in plants with successive cambia are produced by vascular cambia (right-pointing arrows) that become surrounded by conjunctive tissue (left-pointing arrow). In a plant with a conventional single cambium, a vessel at a given point in the shoot becomes ever more distant from the cambium as the shoot grows in girth. Because plants with successive cambia are innervated with cambia, vessels remain in proximity to living phloem for longer periods. *Froelichia humboldtiana* (Amaranthaceae, Caryophyllales). Scale bar (a–c) = 500 μ m; (d–i) = 50 μ m (scale in d, e same as in f).

narrow vessels. As a result, scalariform plates should be increasingly eliminated as shoot length, and therefore vessel diameter, increase, consistent with the lower vessel-diameter–maximum-plant-height scaling slope found by Medeiros et al. (2018) in scalariform-plated species as compared to simple-plated species. If vessels are narrower in species with scalariform perforation plates, then achieving a given flow rate per shoot length would require greater vessel density than in species with simple perforation plates.

In addition to vessels, “dicots” bear imperforate tracheary elements as the most abundant cell type, and we tested for the potential effect of different background elements types on vessel-diameter–shoot-length scaling. Imperforate tracheary elements are, like vessel elements, derived from fusiform cambial initials, but unlike vessel elements, lack perforation plates. Background imperforate tracheary elements are regarded here as including true tracheids, which are thought to be conductive, and non-conductive fiber tracheids and libriform fibers (Fig. 2d–f; our classification follows Carlquist [2001]; see Tables 1, 2 and Rosell et al. [2007] for a discussion of the classification used). One of the most striking correlations in functional wood anatomy is that whenever a species has true tracheids as its background elements, the vessels are solitary (Sano et al. 2011). Just as strikingly, the presence of exclusively solitary vessels can be used to predict the presence of imperforate tracheary elements with abundant bordered pits (Carlquist 1984, Rosell et al. 2007, Pratt et al. 2015). Functionally, this pattern is interpreted as reflecting the effects of selection favoring redundancy of conduction and the avoidance of

air-seeding, i.e., the propagation of embolism from a gas-filled vessel at atmospheric pressure to a functional vessel under negative pressure via the distension of the pit membranes that separate them (Hacke and Sperry 2001, Pratt et al. 2015). When the background elements are non-conductive, vessels are inevitably grouped, which is thought to be the result of selection favoring redundancy in the event of embolization of vessels. Presumably such proximity increases the risk of air seeding. In these cases, conductive background elements would provide alternative conductive paths. As a result, solitary vessels would be favored when alternative routes are available because solitary vessels are presumably less subject to air-seeding. Given that tracheids provide some flow, it could be that species with these elements would have narrower or fewer vessels for a given shoot length than species with non-conductive background elements (libriform fibers and fiber tracheids).

Some species with libriform fibers or fiber tracheids can have one or both of additional and conductive types of imperforate tracheary elements, vasicentric and vascular tracheids (Carlquist 1985, 1987, Rosell et al. 2007, Pratt et al. 2015). Because they are potentially associated with variation in hydraulic behavior, it is possible that species with different imperforate tracheary elements have differing vessel-diameter–shoot-length scaling behavior. Vasicentric tracheids are present in sheaths around vessels or between vessels in vessel groups (often in conspicuous tangential or diagonal, “dentritic,” or “flame-like” vessel groups; Carlquist 1987) in what is otherwise a non-conductive background of fiber tracheids or libriform fibers (Figs. 2g, 3d). In most cases,

TABLE 2. Correspondence of the imperforate tracheary element delimitation used with other delimitations.

Imperforate tracheary element classification used here	Definition followed here (Carlquist 1985, Rosell et al. 2007)	International Association of Wood Anatomists wood identification conventions (IAWA Committee 1986)	Cai et al. (2014) “Fiber bridges”
True tracheid	ITEs with abundant, distinctly bordered pits comparable in size and density to those on lateral vessel walls, thought to be conductive.	Fibers with borders >3 μm (“distinctly bordered pits”); no attempt to distinguish between conductive and non-conductive elements.	Cai et al. refer indistinctly to true tracheids as fibers, fiber tracheids, and groups of ≥1 tracheids separating vessels as “fiber bridges.”
Fiber tracheid	ITEs with pit borders that are narrower or less dense than those on lateral vessel walls, thought to be non-conductive.		Cai et al. do not recognize a non-conductive ITE with distinctly bordered pits category.
Libriform fiber	ITEs with simple pits (i.e., without borders); non-conductive and often living.	Fibers with borders <3 μm.	Cai et al. define these as “fibers without pits” (meaning without bordered pits).
Vasicentric tracheid	Conductive ITE present adjacent to vessels in addition to libriform fibers or fiber tracheids.	Imperforate cells with numerous distinctly bordered pits in their radial and tangential walls, present around the vessels, and different from ground tissue fibers.	Not mentioned.
Vascular tracheid	Conductive ITE present to the exclusion of vessels in latewood in species with fiber tracheids or libriform fibers.	Imperforate cells resembling in size, shape, pitting, and wall ornamentation narrow vessel elements and intergrading with the latter.	Not mentioned.

Note: ITE, imperforate tracheary element.

vasicentric tracheids seem to have evolved from vessel elements, via loss of perforation plates, because they are usually very similar to vessel elements in their wall morphology, staining, and in that, like vessel elements, they usually do not elongate beyond the length of the fusiform cambial initials that gave rise to them. Like true tracheids, vasicentric tracheids are thought to confer an alternative and safer conductive pathway in the event of embolism of the vessels (Barotto et al. 2016). They are very common in sclerophyllous dryland plants. Because their presence is thought to be an adaptation to drought, and because narrow vessels are likely more resistant to embolism than wide ones (Cai and Tyree 2010, Jacobsen et al. 2016, 2019), narrow vessels for a given shoot length would seem plausibly favored in species with vasicentric tracheids. Some species have conductive imperforate elements called vascular tracheids, which are morphologically similar to vasicentric tracheids but are present in a band of latewood that does not include vessels (Fig. 2h), usually in soft-leaved deciduous species (Carlquist 1987, Rosell et al. 2007, Pratt et al. 2015, Venturas et al. 2016, Jacobsen et al. 2016). Vascular tracheids were relatively infrequent in our data, but we also tested for differences in scaling between species with and without vascular tracheids. Like vasicentric tracheids, they are usually found in plants in drought-prone areas, again in areas where selection would plausibly favor relatively narrow vessels for a given shoot length. The diversity of imperforate tracheary elements therefore seems potentially associated with differences in vessel-diameter–shoot-length relationship.

A further major anatomical configuration that could affect vessel-diameter–shoot-length scaling is the presence of a single conventional cambium or successive cambia. Most species of “dicots” have a single vascular cambium that produces secondary phloem to the outside and secondary xylem to the inside. In these plants, the proximity of vessels to the phloem, which provides the photosynthates essential for embolism recovery and respiration (Salleo et al. 2004, 2006, Nardini et al. 2011, Brodersen and McElrone 2013), decreases rapidly as the xylem cylinder expands. Species with successive cambia have vascular cambia throughout the shoot (Fig. 2i; Carlquist 2007). These cambia are produced by an outermost “master cambium” that produces secondary cortex to the outside and vascular cambia plus conjunctive tissue (cells, often parenchyma, that intervene between the products of the vascular cambia) to the inside (Fig. 2i). Species with successive cambia are very widespread globally (e.g., the genus *Atriplex* [Chenopodiaceae] alone is virtually cosmopolitan in warmer parts of the globe), being especially abundant in warm, dry areas (Robert et al. 2011) and in lianas, which are also most abundant in warmer areas (Isnard and Silk 2009, Angyalossy et al. 2012). Judging by the large accumulations of dead phloem often found in species with successive cambia, vessels in these species are likely to remain in proximity to active phloem much longer than in

species with conventional cambia (Fig. 2i). As a result, species with successive cambia could have vessels that are active for much longer than in species with conventional cambia (Kocacinar and Sage 2003, Carlquist 2007, Robert et al. 2011, Rosell et al. 2017b). If the vessels in plants with successive cambia are functional for longer times than in those with conventional ones, then it could be that fewer vessels per square millimeter (lower vessel density) are required as the shoot grows in circumference, and that vessels could be narrower for a given shoot length. If these notions are correct, then the vessel-diameter–shoot-length and the vessel-density–shoot-length intercept of species with successive cambia should be lower than those of species with conventional cambia.

A final major aspect of xylem that has often been implicated in differences in plant hydraulic behavior is wood density (Hacke and Sperry 2001, Jacobsen et al. 2007, Chave et al. 2009, Zanne et al. 2010, Lin et al. 2015, Greenwood et al. 2017). Because species with lower wood density have thinner and less mechanically resistant cell walls (Ziemińska et al. 2013), they are likely more vulnerable to conduit deformation under negative pressure (Hacke et al. 2001). Wall deformation is potentially associated with embolism, because it can lead to distension of interconduit pit membranes and therefore air-seeding (Hacke and Sperry 2001). We tested the prediction that, for a given plant height, species with lower wood densities should have wider vessels.

Our approach allowed us to show that, while vessel diameter scales broadly similarly with shoot length across the various functional groupings examined, there are numerous ecologically significant differences between them. These results illustrate that it is possible to go far in understanding the drivers of variation about the y-axis (variation in mean vessel diameter or vessel density for a given height or shoot length), and through this understanding, identify vectors of selection shaping plant hydraulic systems across habits and climates.

METHODS

Study species

Testing our predictions regarding the ways that different anatomical modes might affect the vessel-diameter–shoot-length relationship across the angiosperms required a large data set covering as many major clades of “dicots” as possible, as well as reflecting appropriate variation in the anatomical features of interest. We included only self-supporting, vessel-bearing, vascular cambium-bearing, non-monocot angiosperms. With this selection, we aimed to maximize comparability to be able to detect the effect, if any, of the variables of interest on the vessel-diameter–shoot-length relationship. We drew on published vessel-diameter–shoot-length data from our previous studies (546 species from Olson and

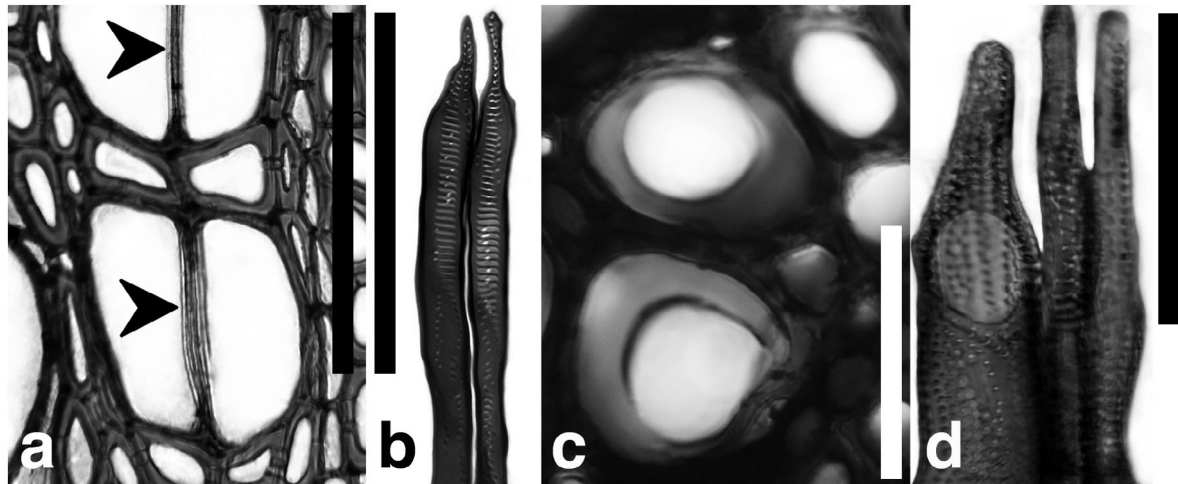


FIG. 3. Photomicrographs of scalariform and simple perforation plates. (a, b) Scalariform perforation plates. (a) In transection, scalariform perforation plates can be seen as bars (arrows), usually oriented radially, that traverse vessels. *Hedyosmum mexicanum* (Chloranthaceae, Chloranthales). (b) In maceration, scalariform perforation plates are readily seen as made up of many slit-like perforations with the vertical series of bars giving a ladder like appearance. *Desfontainia spinosa* (Columelliaceae, Bruniales). (c, d) Simple perforation plates. (c) In transection, simple perforation plates appear as more or less circular holes between vessel elements. The perforation relative to the vessel diameter can sometimes be narrow, as in *Petenaea cordata* (Petenaceae, Huerteales). (d) In maceration, the perforation plate can be seen as an elliptical hole that joined one vessel element to the next member of the vessel. The vessel element (the cell on the left) is flanked by two vasicentric tracheids. *Alvaradoa amorphoides* (Picramniaceae, Picramniales). Scale (a, b) = 200 μm ; (c) = 50 μm ; (d) = 100 μm .

Rosell [2013], Olson et al., 2013, 2014, 2018b, Rosell and Olson [2014]) and added data from 95 species gathered for the present study (data are available from the TRY database *online*).² When the same species was collected at more than one locality, this is designated with an additional column after the species epithet).

To collect samples, we measured shoot length from the base of the trunk to the tip of the distalmost twig, using a flexible tape and following the shoot in the case of sinuous shrubs. For most plants <8 m tall, we used a rigid tape to measure height. For taller plants, we usually used a TruPulse laser rangefinder to measure height. We collected samples from the outer basal-most xylem, above buttresses when present, as well as from the shoot tip distal-most from the shoot base.

We cut thin sections for light microscopy and measured vessel diameter using the ocular micrometer. We made 25 measurements of vessel diameter and vessel density per sample, and most species were represented by three individuals. We scanned haphazardly across growth rings in the outer xylem. We also macerated samples with Jeffrey's fluid (Johansen 1940). We calculated mean values per species and log-transformed all continuous variables. Based on our samples and sections, on our field experience, and on literature sources, we coded as many species as possible for the variables described in the following sections, and are defined in Tables 1 and 2 and illustrated in Figs. 2 and 3.

We coded all species into three commonly used classes, which were ring porous, semi-ring porous, and diffuse

porous (Wheeler et al. 2007). These large categories are known as "porosity types" to distinguish this classification from those of growth rings, which include many more types and make reference to cell types beyond vessels (Carlquist 2001). We classified species into porosity types based on the type exhibited at the base of the shoot, whether or not the porosity type was the same at the shoot tip (in most cases they were the same, but in some cases when the shoot base was ring or semi-ring porous, the tip was diffuse porous, e.g., when the tip samples were collected before the end of the growing season; in no cases were tips ring or semi-ring porous and diffuse at the base; see also Rodriguez-Zaccaro et al., 2019). Porosity types are defined in Table 1 and illustrated in Fig. 2. In species with successive cambia in which xylem rings, plates, or strands were scattered throughout intervening tissue, porosity was classed based on the behavior of the vessels within the vascular areas, regardless of how distant these strands/bands were.

Evergreen species were those that never or only rarely drop their leaves simultaneously. These were mostly sclerophyll species, though we did code as evergreen a few species that are drought deciduous under extreme conditions, but under the usual conditions at the sites where we collected them, they retain their leaves all year round. The annuals *Argemone mexicana* (Papaveraceae) and *Cordylanthus rigidus* (Orobanchaceae) were excluded from leaf phenology analyses. We separated deciduous species in two categories. One was drought deciduous, which consisted of species that lose their leaves largely in response to drought. The second deciduous category was cold deciduous, for species that drop their leaves in

² www.try-db.org

the cold season in freezing-prone locales. We made the drought vs. cold deciduous distinction because the mechanisms of drought vs. freezing-induced embolism are different. Drought induced embolism occurs under excessive xylem tensions, but freezing-induced embolism is caused by gas coming out of solution into the sap and expanding when the xylem comes under tension (Hacke and Sperry 2001, Pittermann and Sperry 2003, Sevanto et al. 2012). These different mechanisms might impose differing selective pressures on vessel diameter given shoot length. Some of our species could not be categorized as evergreen, drought deciduous, or cold deciduous. Some of them were coded as “leafless” to include plants such as *Casuarina* (Casuarinaceae), *Hibbertia juncea* (Dilleniaceae), *Jacksonia* (Fabaceae), or *Tamarix* (Tamaricaceae), which lack leaves but are not succulent. Stem succulents lacking leaves were classed as “stem succulents,” e.g., *Euphorbia lomelii* (Euphorbiaceae), *Pilosocereus purpusii*, and *Stenocereus* spp. (both Cactaceae). This category also included species that only have leaves on new shoots, but are otherwise leafless (*Euphorbia diawlunana* and *E. personata*, both Euphorbiaceae).

Each species was coded as having simple or scalariform perforation plates based on the preponderant perforation plate type at the shoot base (Fig. 3).

We coded three variables to describe imperforate tracheary element types. One of these variables referred to the “ground” or typically most common type, and had three levels, which were true tracheids, fiber tracheids, and libriform fibers (Carlquist 2001, Rosell et al. 2007, Pratt et al. 2015; note that all three of these are referred to as “fibers” in the IAWA Committee [1986] wood identification character list; see Table 2). All of these types usually have intrusive “tails” that result from elongation beyond the lengths of the fusiform cambial initials that give rise to them (Carlquist 2001). True tracheids are densely provided with pits with conspicuous pit chambers (Fig. 2d; the widened area where the pit membrane is, also called a border) and are thought to be conductive (Carlquist 1984, Sano et al. 2011). Fiber tracheids are less densely pitted, and have pits with smaller chambers than those of tracheids, and are thought to be non-conductive (Fig. 2e). Libriform fibers have pits without a widened chamber (Fig. 2f) and are non-conductive. We did not code the two Caricaceae because they have secondary xylem consisting entirely of vessel elements, axial, and ray parenchyma, and lack imperforate tracheary elements. For the species with fiber tracheids or libriform fibers, we created one variable recording presence or absence of vasicentric tracheids, and another for presence or absence of vascular tracheids (see Tables 1, 2 for definitions, and Figs. 2g, h, and 3d). Finally, for analyses of the potential effects of vasicentric and vascular tracheids on vessel diameter scaling, we excluded species with true tracheids, because these species lack vasicentric and vascular tracheids.

We coded a presence/absence successive cambia variable. Species were coded as having successive cambia

when they had vascular cambia throughout the shoot cylinder, each associated with a strand, plate, or ring of secondary xylem and with active and accumulated crushed phloem (Fig. 2i). The outermost layers of the shoots of these species bore periderms overlying a secondary cortex and were phloem free. We did not include intraxylary phloem, which is phloem intruding into the pith from the vicinity of the primary xylem (Carlquist 2001).

We measured wood density using the water displacement method to estimate volume, drying at 100°C (Williamson and Wiemann 2010, Williamson et al. 2012) to measure dry mass, estimating wood density as the ratio between dry mass and fresh volume (g/cm^3). We measured wood density from samples from the basal shoot xylem on portions of the same samples used for anatomical section.

Statistical analyses

To test the effect of porosity type, wood density, leaf phenology, perforation plate type, successive cambia, background imperforate tracheary element type, and vasicentric and vascular tracheids on the scaling of mean vessel diameter at the shoot base (VD_{base}) and shoot tip (VD_{tip}), and of vessel density at the shoot base ($\text{Vmm}_{\text{base}}^2$) and shoot tip ($\text{Vmm}_{\text{tip}}^2$), with shoot length (SL), we used the following general procedure. We fit linear regressions that included $\log(\text{VD})$ or $\log(\text{Vmm}^2)$ as the dependent variable and $\log(\text{SL})$ plus an additional predictor variable, and the corresponding interaction term. The additional predictor variable was categorical, except in the case of wood density, which was continuous. For each model, we first tested the significance of the interaction term. When the variable accompanying shoot length as predictor was categorical, a significant interaction term denoted differences in slope between the different categories. When the interaction term was not significant, this denoted that the scaling slope between the different categories was statistically indistinguishable, and so we re-fit the models without the interaction term. Often the coefficient associated with the categorical variable was significant, indicating similar scaling slope but differing y -intercepts across levels of the categorical variable. In other cases, the coefficient associated with the categorical variable was not significant, and the model simply reduced to the general model of VD or Vmm^2 against shoot length. The procedure for the models including wood density was identical to the general procedure with the exception that density is a continuous variable.

Some of the models departed from this general procedure. One of these was the exploration of the effects of porosity. Because earlywood and latewood in ring porous plants are so markedly different in diameter, and because they are produced in differing ecological situations, with wide earlywood vessels being functional mostly during periods of mild temperatures and abundant water, and narrow latewood vessels being

functional into periods of cold, it seemed possible that they might exhibit differing scaling behaviors. Therefore, for the ring porous species, we tested for differences in scaling based on the 12 widest and 12 narrowest vessels per individual. To provide a maximal contrast with the ring porous species, thought to experience markedly different temperature regimes between early and latewood, we performed a similar test for the low wood density diffuse porous species. In contrast to ring porous species, these are mostly tropical species that do not experience marked temperature differences, and the deciduous ones all tend to drop their leaves early, rather than persisting late into the unfavorable season, and so they offer an ideal contrast to the ring porous species. For this test, we identified the 25% of species with the lowest species mean wood density of the diffuse porous species in our data set, which were the species with a mean density of $\leq 0.42 \text{ g/cm}^3$. Another situation that departed from the general procedure involved species with scalariform perforation plates. We found that VD_{base} in species with scalariform perforation plates scaled with a lower slope than in species with simple perforation plates. At the same time, species with tracheids also scaled with a lower slope than those with libriform fibers or fiber tracheids. Because it is known that scalariform perforation plates are often found in species with tracheids, we tested for this association. When we found the relationship to be significant, we removed scalariform-plated species, and re-fit the model predicting VD_{base} based on shoot length and imperforate tracheary element type without tracheid-bearing species.

Finally, to examine multivariate associations between variables, including categorical and continuous variables, we used the functions PCAmix and PCArot from the R package PCAmixdata (Chavent et al. 2014). PCAmix carries out a principal component analysis (PCA) on data sets with continuous and categorical variables, whereas PCArot rotates mixed data to ease interpretation of principal components (Chavent et al. 2012). We carried out two PCA for mixed data (hereafter PCA) based on two different subsets of our data set. The first PCA analyzed all species and most variables, excluding the presence/absence of vasicentric and vascular tracheids. These variables were left out because they are very strongly negatively associated with background imperforate tracheary elements, being only present when true tracheids are absent, and as a result had a very large effect in the analysis including background imperforate tracheary elements, obscuring the patterns of association between the other variables. The second PCA was applied to a data set including species without true tracheids and with simple perforation plates. This was again because true tracheids are always absent in species with vasicentric or vascular tracheids, and this perfect negative correlation unduly affected the analysis. Excluding species with true tracheids therefore allowed us to probe the relationship between vasicentric and vascular tracheids and porosity, wood density, and phenology. In

both PCAs, we used vessel-diameter–shoot-length residuals instead of the two raw variables to examine the relationship of the anatomical and functional traits with standardized vessel diameter, excluding the potential effect of shoot length on these other traits. We also excluded leafless and stem succulent species from analyses due to their low representation in the data set.

Phylogenetically informed analyses.—To control for potential statistical non-independence between related species, we performed phylogenetic generalized least squares regressions (PGLS) to analyze porosity type, wood density, leaf phenology, perforation plate type, successive cambia, imperforate tracheary element type (in species with simple plates), vasicentric tracheids, and vascular tracheids effects on vessel-diameter– and vessel-density–shoot-length scaling. We built a phylogeny for species in the data set using Soltis et al. (2011), and Chase et al. (2016) for the backbone, assigning branch lengths using the age estimates reported in Table S2 of Magallón et al. (2015) for 45 internal nodes corresponding to family and higher taxonomic levels using the R package ape, with a relaxed clock model. We fit PGLS models by restricted maximum likelihood with a lambda model (λ), using the phylogenetic tree given in Data S1: [OlsonetalPorosityPhylogeny.txt], in the R package caper. For the exploration of ring porosity and low wood density with diffuse porosity, we fit separate models for the “wide” and “narrow” categories, and compared whether there was overlap between parameter estimates and confidence intervals of the opposite category.

RESULTS

Our data set consisted of representatives from 48 orders, 160 families, 641 species, and 1,721 samples of vessel-bearing “dicots” (data are available from the TRY database; see footnote 8). It included 36 ring porous species, 99 semi-ring porous species, and 506 diffuse porous species. 165 species had tracheids as background imperforate tracheary elements, 93 species had fiber tracheids, 381 species had libriform fibers, and 2 species lacked imperforate tracheary elements entirely. Regarding phenological categories, we coded 73 cold deciduous species, 119 drought deciduous ones, and 425 evergreen species. The data set also included 7 leafless species and 17 stem succulents, but because of the low numbers of these categories we removed them from the analyses testing for scaling differences between phenological categories. Of the 474 species with fiber tracheids or libriform fibers, we identified 124 as having vasicentric tracheids and 45 having vascular tracheids. We included 625 species with a single conventional cambium, and 16 species with successive cambia. Wood density values were available for 78% of the species.

When fitting a regression using all of the data and without additional predictor variables, VD_{base} scaled with SL with a slope of 0.430 (95% CI 0.402–0.458), and

TABLE 3. Vessel-diameter– and density–shoot length scaling with porosity types, and in early and latewood of ring porous and low wood density species (≤ 0.42 g/cm³).

Response variable	<i>N</i>	<i>R</i> _{adj} ²	df	<i>F</i>	Slope equality <i>P</i>	Slope inequality <i>P</i>	Slope	Intercept	Fig.
VD_{base}									
~SL	641	0.59	1, 639	899.0***	–	–	0.430 (0.402, 0.458)	1.448 (1.429, 1.467)	4a
~SL + Porosity	641	0.60	3, 637	314.6***	0.089	<0.001	0.416 (0.388, 0.445)	a, ring: 1.470 (1.394, 1.547) a, diffuse: 1.467 (1.446, 1.489) b, semi: 1.386 (1.329, 1.444)	4b
~SL + Wide/Narrow Vessels									
Ring porous species	72 (36 spp.)	0.87	3, 68	159.7***	<0.001	<0.001	a, wide: 0.529 (0.361, 0.697) b, narrow: 0.307 (0.237, 0.376)	wide: 1.556 (1.435, 1.678) narrow: 1.305 (1.254, 1.355)	4c
Low wood density and diffuse porous species	266 (133 spp.)	0.71	2, 263	317.9***	0.680	<0.001	0.469 (0.425, 0.513)	a, wide: 1.644 (1.559, 1.730) a, narrow: 1.327 (1.286, 1.368)	4d
VD_{tip}									
~SL	536	0.23	1, 534	162.0***	–	–	0.196 (0.165, 0.226)	1.060 (1.039, 1.081)	4j
~SL + Porosity	536	0.26	3, 532	63.59***	0.316	<0.001	0.181 (0.150, 0.211)	a, diffuse: 1.085 (0.972, 1.198) a, b, ring: 1.041 (0.984, 1.097) b, semi: 0.995 (0.874, 1.116)	4k
~SL + Wide/Narrow Vessels									
Ring porous species	66 (33 spp.)	0.57	2, 63	43.75***	0.661	<0.001	0.173 (0.110, 0.236)	a, wide: 1.157 (1.028, 1.286) b, narrow: 0.891 (0.832, 0.950)	4l
Low wood density and diffuse porous species	244 (122 spp.)	0.60	2, 241	183.8***	0.197	<0.001	0.256 (0.211, 0.301)	a, wide: 1.275 (1.188, 1.362) b, narrow: 0.921 (0.880, 0.963)	4m
Vmm_{base}²									
~SL	615	0.40	1, 613	408.2***	–	–	–0.741 (–0.813, –0.669)	2.209 (2.159, 2.258)	5a
~SL + Porosity	615	0.43	3, 611	156.1***	0.56	<0.001	–0.694 (–0.766, –0.622)	a, diffuse: 2.135 (1.186, 2.412) a, ring: 2.211 (2.074, 2.349) b, semi: 2.416 (2.122, 2.711)	5b
~SL + Wide/Narrow Vessels									
Ring porous species	72 (36 spp.)	0.66	3, 68	47.57***	<0.005	<0.001	a, wide: –1.216 (–1.866, –0.567) b, narrow: –0.628 (–0.897, –0.358)	wide: 1.987 (1.517, 2.456) narrow: 2.371 (2.177, 2.566) not fit because many of the fields of vessel density had zero vessels	5c
Low wood density and diffuse porous species									
Vmm_{tip}²									
~SL	480	0.07	1, 478	34.02***	–	–	–0.176 (–0.236, –0.117)	2.969 (2.930, 3.008)	5j
~SL + Porosity	480	0.10	3, 476	17.08***	0.59	<0.001	–0.152 (–0.212, –0.093)	a, diffuse: 2.932 (2.721, 3.142) a, b, ring: 2.961 (2.856, 3.066) b, semi: 3.076 (2.852, 3.301)	5k

TABLE 3. Continued.

Response variable	<i>N</i>	R^2_{adj}	df	<i>F</i>	Slope equality <i>P</i>	Slope inequality <i>P</i>	Slope	Intercept	Fig.
~SL + Wide/Narrow Vessels	62 (31 spp.)	0.22	2, 59	9.50***	0.744	<0.001	-0.097 (-0.219, 0.025)	a, wide: 2.763 (2.506, 3.020) b, narrow: 3.049 (2.933, 3.165) not fit because many of the fields of vessel density had zero vessels	5l
Low wood density and diffuse porous species									

Notes: Porosity, porosity types; SL, shoot length; VD_{base} , vessel diameter at shoot base; VD_{tip} , vessel diameter at shoot tip; Vmm^2_{base} , vessel density at shoot base; Vmm^2_{tip} , vessel density at shoot tip. Types of porosity: diffuse porous, diffuse; ring porous, ring; semi-ring porous, semi. *** $P < 0.001$; when intercepts differ but slopes are identical, we label the statistically significant groupings of intercepts with letters to the left of the coefficients; we use similar notation only on the slopes when these differ. Dashes in cells indicate parameters that were absent in a given model.

VD_{tip} with a slope of 0.196 (95% CI 0.165–0.226; Table 3), well within the range of previously recovered slopes (Anfodillo et al. 2006, Petit et al. 2008, Bettati et al. 2012, Olson et al. 2014, 2018b, Petit and Crivellaro 2014, Rosell and Olson 2014). Vessel density (Vmm^2) scaled with SL with a slope of -0.741 (95% CI -0.813 to -0.669) at the shoot base and -0.176 (95% CI -0.236 to -0.117) at the shoot apex (Table 3). The general VD_{base} -SL fit is shown in Fig. 4a, with the fits including the other variables shown in Figs. 4b–i. The general VD_{tip} -SL fit is shown in Fig. 4j, with the specific fits for the different subgroups of species being shown in Figs. 4k–r. In Fig. 4a, j, points with different colors represent the different porosity types.

Vessel density scaled with a negative slope, with fewer vessels per unit area at higher SL (Fig. 5a). Because higher SL corresponds to higher VD, the negative relationship between VD and SL was expected given the well-documented trade-off between Vmm^2 and VD (Carlquist 1975, Zanne et al. 2010, Martinez-Vilalta et al. 2012, Rosell and Olson 2014, Pratt and Jacobsen 2017). The Vmm^2 -SL relationship mirrored the VD-SL one in that higher VD-SL intercepts, which denote wider vessels for a given height, had fewer vessels per square millimeter of transection (lower vessel density), and thus lower Vmm^2 -SL intercepts (Tables 3–5). The fits for Vmm^2 -SL of the species subgroups based on the different anatomical and functional features are shown in Fig. 5b–r.

Both at the shoot base and the tip, non-significant SL × porosity type interaction terms ($P = 0.089$ and 0.316, respectively) indicated that the VD-SL scaling slope was similar across porosity types at the shoot base and tip, though they did scale with differing intercepts (Table 3, Fig. 4b, k). These differing intercepts indicated that, for a given shoot length, semi-ring porous species had narrower mean vessel diameters than diffuse or ring porous species. Ring and diffuse porous species had similar mean vessel diameters for a given height (Table 3). As expected, these differences in VD-SL intercept were paralleled by differences in Vmm^2 -SL intercepts (Table 3, Fig. 5b, k). Semi-ring porous species, which had the narrowest mean vessel diameters for a given shoot length, had the highest vessel density given SL and thus the highest intercept, which differed statistically from the other two porosities.

Separating the 12 widest (earlywood) and 12 narrowest (latewood) vessels per individual of the ring porous species, and examining the scaling of earlywood and latewood separately, we found different scaling slopes between the wide earlywood vessels and narrow latewood ones at the shoot base (Table 3, Fig. 4c). Earlywood vessels scaled with a slope of 0.529 whose confidence interval (0.361–0.697) included the general slope (0.430), but the latewood ones scaled conspicuously lower, with a slope of 0.307 and a 95% confidence interval (0.237–0.376) that did not include the general slope. This result suggested two possibilities. One is that

the narrowest vessels, regardless of when they are produced in a season or in a growth ring, scale with a lower slope than widest vessels. The other possibility is that late in the season, when the latewood vessels are the main conducting elements, increasingly narrow vessels are favored relative to plant height as compared to conditions early in the season. We distinguished between these two possibilities by testing for differences in scaling between the 12 widest and 12 narrowest vessels in the 133 species with diffuse porosity representing the lowest quartile in wood density values. Low wood density species tend to be fugaciously deciduous (i.e., drop their leaves at the first sign of drought (Méndez-Alonzo et al. 2012)) and in our data were all from frost-free or nearly frost-free locales. Unlike in ring porous species, in low wood density species wide and narrow vessels are distributed throughout a given area of transection. In these species, the non-significant wide/narrow \times SL interaction term indicated that both wide and narrow vessels scale with the same slope (both at the base and at the tip), unlike in ring porous species (Table 3, Fig. 4d, m), suggesting important differences in the way that selection acts on vessel diameter distribution between these porosity types.

Taking shoot length into account, wood density as a continuous variable was significantly and negatively associated with vessel diameter. That is, given plants of the same shoot length but differing wood density, those with lower wood density had predictably wider vessels, both at the shoot tip and the base (Table 4).

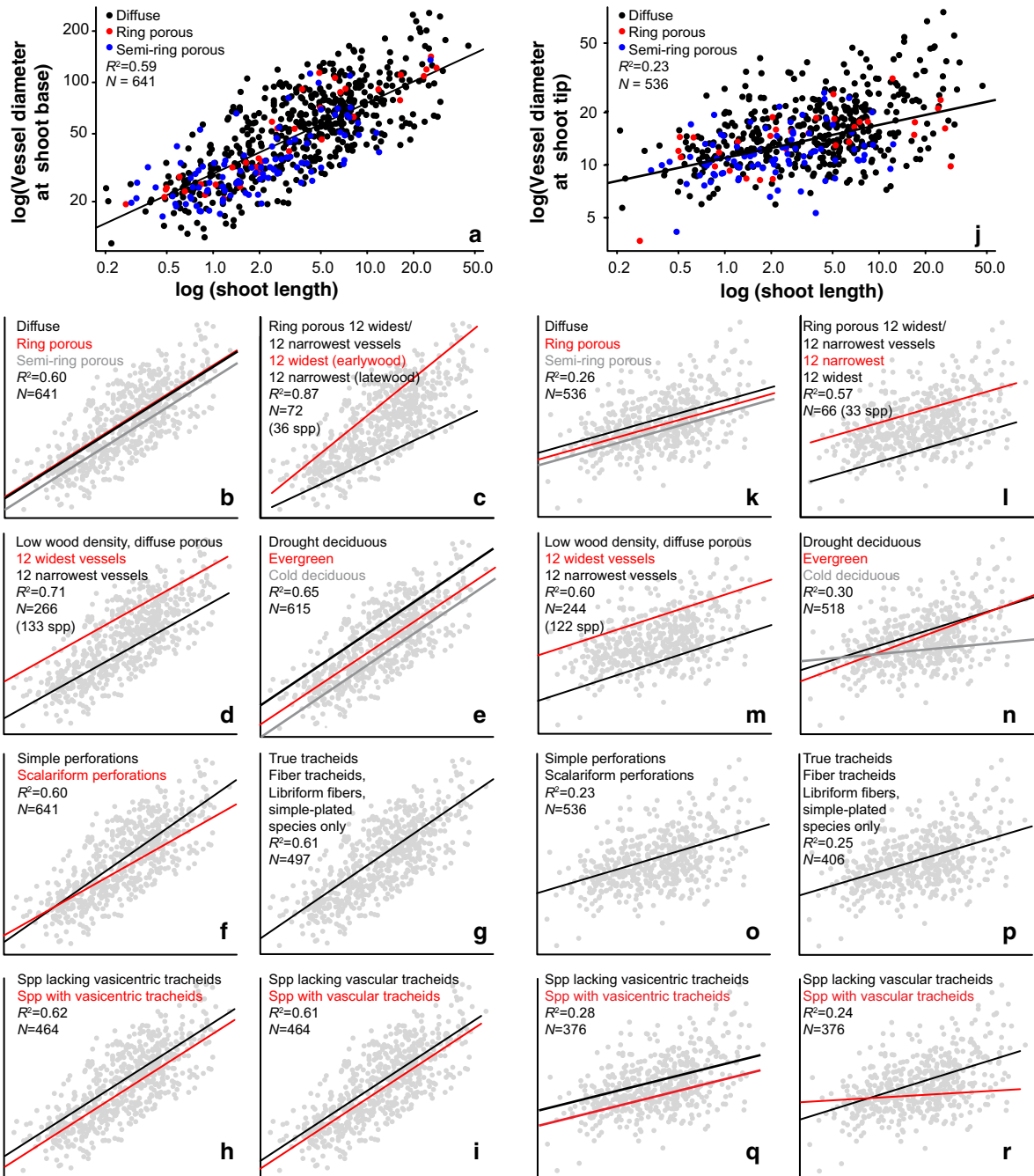
Removing the stem succulent and leafless species, plus the two annual species, left a total of 615 species for which VD_{base} data were available, and these were categorized as drought deciduous, evergreen, or cold deciduous. Intriguingly, different patterns were observed at the shoot tip and the base. With regard to VD_{base} and Vmm^2_{base} , the leaf phenology \times SL interaction term was non-significant, indicating similar scaling slopes across all three phenological categories. Intercepts showed that drought deciduous species had the widest vessels for a given height, with the cold deciduous species having the narrowest ones (Fig. 4e). At the shoot tip, we observed different slopes across the phenological categories. Drought deciduous species scaled broadly similarly to the general fit with regard to both VD_{tip} (Fig. 4n) and Vmm^2_{tip} (Fig. 4n). However, cold deciduous species scaled with a strikingly lower VD_{tip} –SL slope, and a correspondingly shallow Vmm^2_{tip} –SL slope (Table 4, Figs. 4n, 5n).

Both VD_{base} and Vmm^2_{base} scaled with different slopes in scalariform vs. simple-plated species (Table 4). With increasing plant height, mean VD_{base} for a given height was increasingly different between perforation plate types. Species with scalariform perforation plates had markedly narrower vessels (Fig. 4f), which were more dense (Fig. 5f), than species with simple plates (Table 4). We did not detect differences in scaling or intercept in VD_{tip} –SL or Vmm^2_{tip} –SL scaling between species with simple and scalariform perforation plates (Figs. 4o, 5o).

With regard to species with successive cambia, we found that neither the *successive* SL interaction nor the *successive* term was significant in models predicting VD_{base} and VD_{tip} (Table 4). This means that species with successive cambia do not have significantly narrower vessels for a given shoot length than those with conventional single cambia, rejecting one of our predictions. However, at the shoot base (Fig. 6a) and at the tip vessel density was significantly lower in species with successive cambia (Table 4). Based on this result, we fit a model predicting Vmm^2 based on VD_{base} plus the *successive* categorical variable. We found that for a given VD_{base} , species with successive cambia have significantly lower Vmm^2 than species with conventional single cambia (Fig. 6b, $N = 615$, $R^2 = 0.72$, $F_{2,612} = 791.1$, $P < 0.001$; slope equality test $P = 0.308$; intercept equality test $P < 0.001$; slope -1.780 (-1.868 , -1.692); intercept successive cambia 4.503 (4.212 , 4.794), non-successive 4.788 (4.640 , 4.937)). The same was observed for VD_{tip} ($N = 479$, $R^2 = 0.59$, $F_{2,476} = 345.0$, $P < 0.001$; slope equality test $P = 0.141$; intercept equality test $P < 0.01$; slope -1.312 (-1.411 , -1.212); intercept successive cambia 4.232 (4.011 , 4.453), non-successive 4.390 (4.274 , 4.505)). So, while species with successive cambia do not differ from species with conventional cambia in their VD –SL relationship, they do have fewer vessels than expected both given shoot length as well as vessel diameter.

The significant $ITE \times SL$ interaction term indicated that species with different imperforate tracheary element (ITE) types scaled with different slopes (Table 5). Specifically, species with tracheids scaled with a slope that was significantly lower than those with tracheids or fiber tracheids; correspondingly, species with tracheids also had higher vessel density than those with non-conductive ITEs (Table 5, Figs. 4g, 5g). However, tracheids have long been known to be commonly associated with scalariform perforation plates (Metcalf and Chalk 1957). Given that species with scalariform perforation plates had a lower VD_{base} –SL scaling slope (Table 4, Fig. 4f), it seemed possible that the lower slope of species with tracheids might be due to this association. To examine this possibility, we first checked for an association between scalariform perforation plates and tracheids, and confirmed this expectation ($\chi^2_{(2)} = 188.2$, $P < 0.001$). Given the association, we removed the species with scalariform perforation plates, and fit the model again (Table 5). Across the simple-plated species, at both the shoot base and tip, in the VD –SL scaling there was no difference in slope or intercept between species with different ITEs (Figs 4g, p). The same was observed for Vmm^2 –SL scaling (Fig. 5g, p).

Species with vascular and vasicentric tracheids behaved similarly to one another. With regard to vasicentric tracheids, we observed different intercepts, though similar VD –SL and Vmm^2 –SL scaling slopes, between species with and without these cells (Table 5).



Species without vascentric tracheids had consistently wider vessels and lower vessel density for a given length than species with these cells (Table 5, Figs. 4h, q, 5h, q). Models with vascular tracheids behaved similarly, with the exception that in species with vascular tracheids, VD_{tip} scaled with a much lower slope than those without (Table 5, Figs. 4i, r, 5i, r).

Regressions using phylogenetic generalized least squares were identical in their biological implications to analyses based on data, with parameter estimates

differing only slightly from non-phylogenetic analyses (Appendix S1).

Multivariate patterns of association

The PCA for mixed quantitative and categorical data revealed strong patterns of multivariate association between variables. In the first PCA, which included most species and excluded vascentric and vascular tracheids, the first three principal components explained 45.5% of

FIG. 4. Vessel diameter (VD; measured in μm)–shoot length (SL; measured in m) scaling at the shoot base (VD_{base}) and the tip (VD_{tip}). (a–i) VD_{base} –SL scaling; each point is a species mean value, and lines are fits from linear regressions. See Tables 3–5 for regression details. (a) General VD_{base} –SL fit with all the data. Colors represent porosity types. (b). Based on species means, all porosity types scaled with the same slope, though with slightly differing intercepts. (c) The 12 narrowest (latewood) vessels scaled with a lower slope than the 12 widest (earlywood) vessels in ring porous species. (d) In contrast, the 12 narrowest and 12 widest vessels scaled with the same slope in the low wood density species ($\leq 0.42 \text{ g/cm}^3$). (e). Drought deciduous species had wider vessels for a given height, cold deciduous narrower ones. (f) Species with scalariform perforation plates scaled with a significantly lower slope than those with simple plates, such that diameters were similar at small shoot lengths, but in larger plants, vessels with scalariform perforation plates were narrower with respect to those with simple plates. (g) When analyzing all species, those with tracheids had a lower slope (not shown; see Table 5). However, scalariform-plated species often have tracheids. When removing species with scalariform plates, no difference in slope or intercept was observed across species with different imperforate tracheary element types. (h, i) Species with vasicentric and vascular tracheids tended to have slightly narrower vessels for a given shoot length as compared to those lacking these conductive imperforate tracheary element types. (j–r) VD_{tip} –SL scaling. (j) General VD_{tip} –SL fit with all the data. Colors represent porosity types. (k) As at the shoot base, based on species means, all porosity types scaled with the same slopes, though with slightly differing intercepts. (l) The 12 narrowest (latewood) vessels scaled with a similar slope as the 12 widest (earlywood) vessels in ring porous species, in contrast to the base. (m) As at the base, the 12 narrowest and 12 widest vessels scaled with the same slope in low wood density species. (n) In contrast to the base, cold deciduous species had a significantly lower slope at the shoot tip, indicating disproportionately narrower vessels in taller cold deciduous species. (o) In contrast to the base, at the shoot tip, species with scalariform perforation plates scaled with identical slopes and intercepts as those with simple plates. (p) Similar to the base, species with different imperforate tracheary element types scaled with similar slopes and intercepts at the shoot tip. (q) As at the base, at the shoot tip, species with vasicentric tracheids had narrower vessels for a given height than those without. (r) Over the majority of the range in shoot length, at the shoot tip species with vascular tracheids had narrower vessels for a given height, though the scaling lines cross at small shoot lengths. Gray points in graphs b–i and k–r show all data; sample sizes and coefficients of determination are given for each graph.

the observed variation (Table 6). Perforation plate type and background imperforate tracheary element (ITE) had high squared loadings in the first dimension, the two continuous variables (wood density and VD–SL residuals) had high loadings in the second dimension, and leaf phenology and growth ring type in the third one (Table 6, Fig. 7). The direction of the strong association between variables indicated by the high squared loadings can be observed in Fig. 7. The second PCA, which included species without true tracheids, showed similar patterns of association as the first PCA. In this second PCA, the first three dimensions explained 51.1% of the observed variance. The squared loadings indicated that the variables growth ring type, presence/absence of vasicentric tracheids, and also of vascular tracheids, were closely associated with the first dimension, and between one another. Porosity type was also associated with leaf phenology in the second dimension. In turn, leaf phenology was also associated with the third dimension, for which the continuous variables wood density and VD–SL residuals also had high loadings (Table 6, Fig. 7). Together, these show that species with lower density wood have higher VD–SL residuals, tend to have libriform fibers, be diffuse porous, and be drought deciduous. Species with high-density wood tend to have lower VD–SL residuals and to be evergreen. Ring porous and semi-ring porous species tend to have higher wood density. Vasicentric and vascular tracheids are associated with semi-ring porosity, higher wood density, and evergreenness.

DISCUSSION

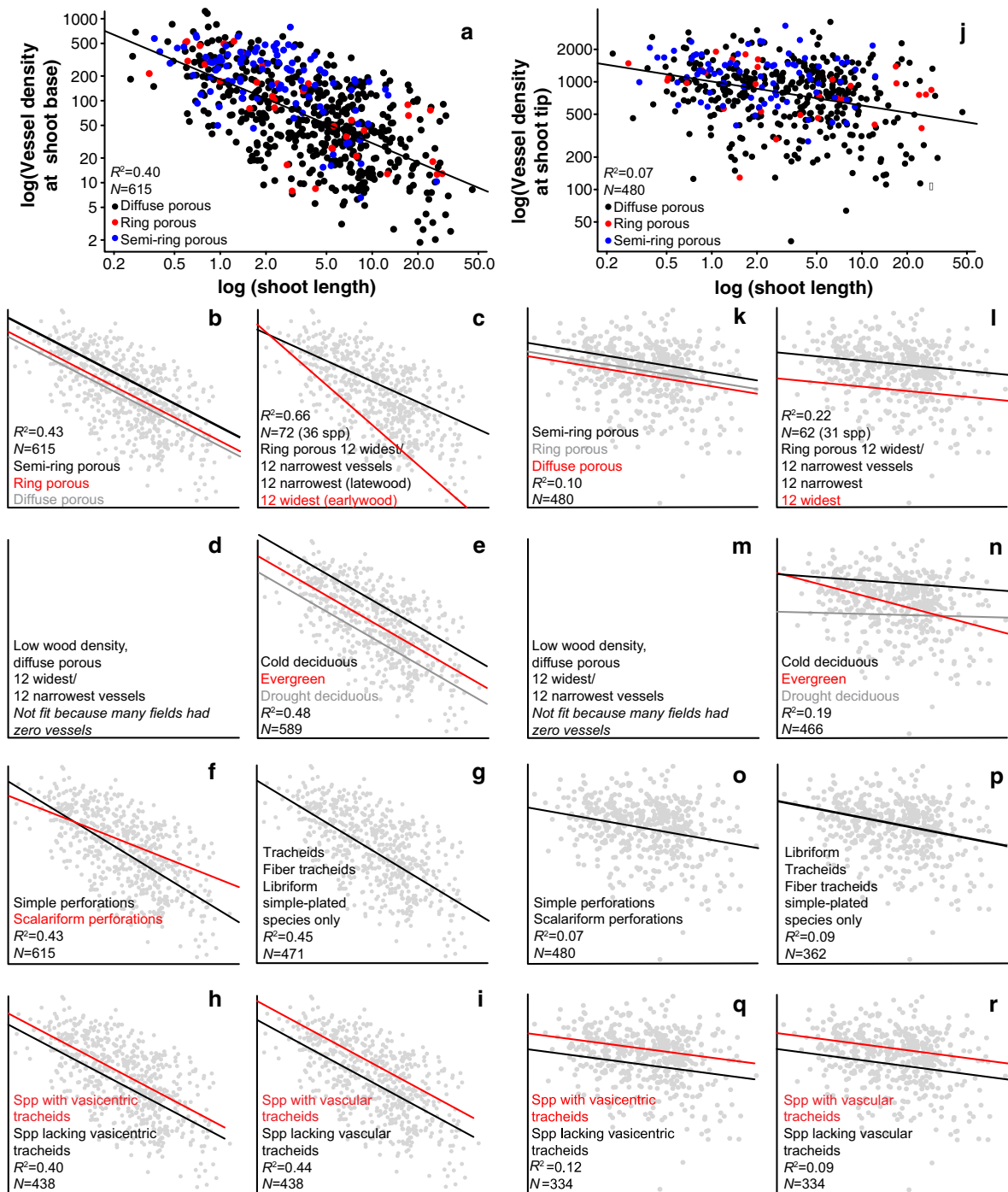
Variance about the y-axis in the VD–SL relationship is rich with biological signal

Our results, together with others (Olson et al. 2014, 2018b, Medeiros et al. 2018, Rosell and Olson 2014,

Morris et al. 2018, Echeverría et al. 2019, Liu et al. 2019), show that the variation about the y-axis in the vessel-diameter–shoot-length relationship (Fig. 1) is in large part not simply statistical noise but important biological signal. It is clear that, while shoot length is the variable that explains by far most of the variance in mean conduit diameter across species, there is still some leeway for selection to favor slightly narrower or wider vessels for a given plant height in different ecological situations (cf. Edwards et al. 2014). Moreover, the results we present here suggest that there is a relatively small number of organizing trait constellations that account for many of these relationships. Aspects that appear to help explain the effects of multiple traits on the VD–SL relationship include wood density and its effects on embolism resistance. Other traits appear relatively independent in their effects on the VD–SL relationship, such as the association between scalariform perforation plates and true tracheids. We first discuss traits that were related to one another in their effects on the VD–SL relationship, before turning to the more isolated traits, and finally implications for these findings for thinking regarding the hydraulic system during plant adaptation to climate and habit diversification.

Wood density as an organizing variable: porosity, leaf phenology, and vasicentric and vascular tracheids

Variation in VD–SL scaling with porosity type, leaf phenology, and vasicentric and vascular tracheids involved variation in wood density (Table 6, Fig. 7). In general, species with lower wood density tended to have, for a given shoot length, wider vessels and were often drought deciduous. This difference in mean vessel diameter given shoot length likely involves the tendency for species with lower wood density species to be more vulnerable to embolism than species with higher wood



density (Hacke et al. 2001, Jacobsen et al. 2007, Chen et al. 2009, Christoffersen et al. 2016, Greenwood et al. 2017). Thinner cell walls mean that low-density species have conduits that are more readily deformed under negative pressure, especially if a vessel with an intact conductive stream under tension is adjacent to an embolized vessel at atmospheric pressure. This deformation could lead to distension of the intervessel pit membranes, widening of pit membrane pores, and entry of gas into

the functional vessel, breaking its water column and blocking the vessel with a gas embolism (Hacke and Sperry 2001, Hacke et al. 2001, Lens et al. 2013). In species of low wood density, natural selection appears to favor avoidance of highly negative tensions (Méndez-Alonzo et al. 2012, Wolfe et al. 2016), and so these species tend to drop their leaves early in response to drought (Chen et al. 2009, Méndez-Alonzo et al. 2013). Fugacious deciduousness means that the vessels are never

FIG. 5. Vessel density (vessels/mm² of xylem transection)–shoot length (SL) scaling at the shoot base (Vmm_{base}^2) and the tip (Vmm_{tip}^2). (a–i) Vmm_{base}^2 –SL scaling. See Tables 3–5 for regression details. (a) General Vmm_{base}^2 –SL fit with all the data. Colors represent porosity types. (b) Vmm_{base}^2 –SL scaling patterns are a mirror image of VD scaling, with semi-ring porous species, which had the relatively narrowest vessels for a given height, having the highest vessel density. (c) Likewise, the Vmm_{base}^2 of the 12 highest vessel count fields (latewood) scaled with a steeper (more negative) slope than the 12 lowest (earlywood) ones in ring porous species. (d) We did not fit a model with the 12 highest and 12 lowest vessel count fields in the low wood density species because in many cases the 12 fields with the lowest vessel density lacked vessels. (e) Drought deciduous species had fewer vessels/mm² for a given height, whereas cold deciduous had more. (f) Species with scalariform perforation plates scaled with a significantly shallower (less negative) slope than those with simple plates, such that at small shoot lengths vessel densities were similar, but in larger plants those with scalariform perforation plates had increasingly higher numbers of vessels/mm² with respect to those with simple plates. (g) When analyzing all data, species with differing imperforate tracheary elements had different slopes (not shown; see Table 5). Across species with simple perforation plates (removing species with scalariform plates), no difference in slope or intercept was observed across species with different imperforate tracheary element types, shown here. (h, i) Species with vasicentric and vascular tracheids, with their narrower vessels, had higher vessel densities than those lacking these conductive imperforate tracheary elements. (j–r) Vmm_{tip}^2 –SL scaling. (j) General Vmm_{tip}^2 –SL fit with all the data. Colors represent porosity types. (k) As at the shoot base, based on species means, all porosity types scaled with the same slopes, though with slightly differing intercepts. (l) In contrast to the base, the 12 highest vessel count fields (latewood) scaled with a similar slope as the 12 lowest vessel count fields (earlywood) in ring porous species. (m) We did not fit a model with the 12 highest and 12 lowest vessel count fields in the low wood density species because in many cases the 12 fields with the lowest vessel density lacked vessels. (n) Cold deciduous species had significantly more vessels/mm² at the shoot tip, drought deciduous fewer, with evergreen species having a steeper (more negative) slope. (o) Vessel density in scalariform-plated species scaled identically as in species with simple plates at the shoot tip. (p) Species with different imperforate tracheary element types scaled with similar slopes and intercepts at the shoot tip, even when scalariform-plated species were removed. (q, r) As at the shoot base, species with vasicentric and vascular tracheids, with their narrower vessels, had higher vessel densities than those lacking these conductive imperforate tracheary elements. Gray points in graphs b–i and k–r show all data.

exposed to highly negative xylem tensions, allowing for very low wood density and low resistance to embolism. Higher wood densities are associated with greater abilities of conduits to resist deformation and air-seeding (Hacke et al. 2001). Because within individual shoots narrower vessels better resist embolism, it is to be expected that selection favoring embolism resistance in evergreen drought- or cold- resistant species would simultaneously favor narrow vessels and high wood density (Hacke et al. 2001, Jacobsen et al. 2007, Greenwood et al. 2017). In our data, drought deciduous species always had simple perforation plates, expected if selection favors simple plates in situations of high variation in peak conductive rates (Olson 2014, Carlquist 2018). They also virtually always had libriform fibers, which are often living and involved in water and starch storage (Carlquist 2014). Selection favoring storage, high peak conductance, and avoidance of highly negative xylem tensions is therefore consistent with our observation of low wood density, libriform fibers, simple perforation plates, and relatively wide vessels in drought deciduous species.

On the opposite extreme of the spectrum of wood density, the same processes favor a different set of features (Table 6, Fig. 7). High wood density species tend to have relatively narrow vessels for a given shoot length, a combination likely favored because both high wood density (via thick vessel and imperforate tracheary element walls) and narrow vessels confer embolism resistance (Hacke et al. 2001, Jacobsen 2005, Cai and Tyree 2010, Jacobsen et al. 2016, 2019). Vasicentric and vascular tracheids are often found in Mediterranean shrubs, vasicentric tracheids often in sclerophyll evergreens, and vascular tracheids in malacophyllous species, that is, species with relatively soft leaves that

tend to wither, though remaining attached to the plants, as the dry season progresses (Carlquist 1985, 1987, Pratt et al. 2015). In turn, species with vascular and vasicentric tracheids are often semi-ring porous (Fig. 7). A conspicuous syndrome on the opposite end of the low wood density-relatively wide vessels-drought deciduous-libriform fibers syndrome is thus high wood density, relatively narrow vessels, evergreen leaf phenology, and libriform fibers or fiber tracheids plus vasicentric and/ or vascular tracheids (Fig. 7). In evergreen Mediterranean species, selection would favor high wood density in resisting highly negative xylem tensions, as well as vasicentric tracheids as highly embolism-resistant conduits in these species in which leaves persist through a long dry season (Jacobsen 2005, Pratt et al. 2015, Jacobsen et al. 2016).

A distinct syndrome at the relatively high wood density end of the continuum was the association between cold deciduousness and ring porosity, a well-known association (e.g., visible in the data of Wheeler et al. 2007) that also emerged in our data. Ring porous species are mostly found in the cold-prone north temperate zone, where freezing would act strongly against the lowest wood densities. Lower wood densities are selected against in cold situations, in part as a result of their vulnerability to freezing-induced deformation of thin cell walls or the forcing of the contents of living cells into adjacent vessels (Martínez-Vilalta and Pockman 2002, Ball et al. 2006, Sevanto et al. 2012). Indeed, in our data species with very low wood densities were only present in non-freezing habitats (data are available from the TRY database; see footnote 8). Via its association with different levels of storage and embolism resistance, wood density thus appears to be involved in the largest suite of associations that we found, involving porosity, leaf

TABLE 4. The effects of wood density, leaf phenology, perforation plate type, and successive cambia on vessel-diameter– and vessel-density–shoot-length scaling.

Response variable	N	R ² _{adj}	df	F	Slope equality P	Slope inequality P	Slope	Intercept	Fig.
VD _{base}	–	–	–	–	–	–	–	–	–
~SL + wood density	501	0.65	2, 498	465.4***	0.184	–	SL: 0.431 (0.402, 0.461) density: –0.339 (–0.439, –0.239)	1.625 (1.565, 1.684)	not shown
~SL + leaf phenology	615	0.65	3, 611	373.3***	0.413	<0.001	0.434 (0.407, 0.461)	a, drought deciduous: 1.569 (1.518, 1.620) b, evergreen: 1.427 (1.407, 1.445) c, cold deciduous: 1.346 (1.288, 1.404)	4e
~SL + perforation	641	0.60	3, 637	324.4***	<0.05	<0.001	a, simple: 0.456 (0.327, 0.585) b, scalariform: 0.367 (0.306, 0.428)	simple: 1.452 (1.357, 1.548) scalariform: 1.426 (1.380, 1.472)	4f
~SL + cambia	641	0.58	–	see VD ~ SL	0.904	0.444	see VD ~ SL	see VD ~ SL	not shown
VD _{tip}	–	–	–	–	–	–	–	–	–
~SL + wood density	457	0.37	3, 453	88.79***	<0.005	–	SL: 0.387 (0.266, 0.508) density: –0.199 (–0.361, –0.037) SL × density: –0.378 (–0.604, –0.153)	1.169 (1.078, 1.259)	not shown
~SL + leaf phenology	518	0.30	5, 512	45.97***	<0.001	<0.001	a, drought deciduous: 0.201 (0.060, 0.343) a, evergreen: 0.237 (0.203, 0.271) b, cold deciduous: 0.060 (0.057, 0.178)	drought deciduous: 1.099 (1.004, 1.194) evergreen: 1.049 (1.027, 1.072) cold deciduous: 1.050 (0.962, 1.138)	4n
~SL + perforation	536	0.23	–	see Vd _{tip} ~ SL	0.430	0.727	see VD _{tip} ~ SL	see VD _{tip} ~ SL	4o
~SL + cambia	536	0.23	–	see Vd _{tip} ~ SL	0.462	0.196	see VD _{tip} ~ SL	see VD _{tip} ~ SL	not shown
Vmm ² _{base}	–	–	–	–	–	–	–	–	–
~SL + wood density	500	0.48	2, 497	230.3***	0.362	–	SL: –0.762 (–0.837, –0.688) density: 0.710 (0.457, 0.964)	1.853 (1.703, 2.003)	not shown
~SL + leaf phenology	589	0.48	3, 585	180.9***	0.057	<0.001	–0.776 (–0.846, –0.706)	a, drought deciduous: 2.000 (1.911, 2.089) b, evergreen: 2.236 (2.059, 2.413) c, cold deciduous: 2.558 (2.348, 2.768)	5e
~SL + perforation	615	0.43	3, 611	152.9***	<0.005	<0.001	a, simple: –0.821 (–1.147, –0.494) b, scalariform: –0.533 (–0.687, –0.379)	simple: 2.206 (1.964, 2.449) scalariform: 2.218 (2.103, 2.333)	5f
~SL + cambia	615	0.40	2, 612	208.3***	0.373	<0.05	–0.740 (–0.812, –0.669)	conventional: 2.215 (2.165, 2.265) successive: 1.968 (1.710, 2.226)	6a
Vmm ² _{tip}	–	–	–	–	–	–	–	–	–
~SL + wood density	411	0.17	3, 407	28.09***	<0.001	–	SL: –0.621 (–0.872, –0.369) density: 0.118 (–0.205, 0.442) SL × density: 0.834 (0.363, 1.304)	2.912 (2.734, 3.090)	not shown

TABLE 4. Continued.

Response variable	N	R^2_{adj}	df	F	Slope equality P	Slope inequality P	Slope	Intercept	Fig.
~SL + leaf phenology	466	0.19	5, 460	22.91***	<0.01	<0.001	a, cold deciduous: -0.073 ($-0.204, 0.059$) a, drought deciduous: -0.025 ($-0.390, 0.340$) b, evergreen: -0.261 ($-0.540, -0.018$)	cold deciduous: 3.130 (3.026, 3.325) drought deciduous: 2.774 (2.504, 3.044) evergreen: 2.993 (2.776, 3.211)	5n
~SL + perforation	480	0.07	—	see $Vmm^2_{\text{tip}} \sim SL$	0.084	0.837	see $Vmm^2_{\text{tip}} \sim SL$	see $Vmm^2_{\text{tip}} \sim SL$	5o
~SL + cambia	480	0.08	2, 477	22.17***	0.103	<0.005	-0.177 ($-0.236, -0.118$)	conventional: 2.976 (2.937, 3.015) successive: 2.724 (2.526, 2.922)	not shown

Notes: Density, wood density; SL, shoot length; VD_{base} , vessel diameter at shoot base; VD_{tip} , vessel diameter at shoot tip; Vmm^2_{base} , vessel density (vessels/mm²) at shoot base; Vmm^2_{tip} , vessel density (vessels/mm²) at shoot tip. Notation as for Table 1.

phenology, imperforate tracheary element types, and perforation plate type.

Suites of covariation not associated with variation in wood density

We detected two smaller suites of covariation that were largely uncorrelated with the wood density-related suite summarized above (Table 6, Fig. 7). One of these small suites involved successive cambia and the other the scalariform perforation plate–true tracheid association. Species with successive cambia all had simple perforation plates and were all diffuse porous, just as the drought deciduous species with single cambia tended to have. Like drought deciduous species, species with successive cambia live in areas with highly fluctuating water availability (Robert et al. 2011), selecting for high peak conductive rates, in which simple plates are favored (Carlquist 2018). But other than this clear association, species with successive cambia varied largely independently of the wood-density-centered suite of variation. Species with successive cambia spanned a wide range of wood densities (0.23–0.71 g/cm³), and most of them were evergreen (12 evergreen, 4 drought deciduous), so they did not appear to be conspicuously aligned along the density–phenology relationship that species with conventional cambia describe. The other small suite of trait covariation not clearly correlated with the wood density-related suite was that involving scalariform perforation plates and true tracheids. These species spanned a wide range of wood densities, from 0.3 to 0.8 g/cm³, with the tendency for scalariform plates to be associated with true tracheids being observed across the range, and thus largely independent of wood density (Table 6, Fig. 7). We now examine these two suites in more detail.

Successive cambia.—Our data rejected the prediction that species with successive cambia should have narrower mean vessel diameter for a given height, but species with successive cambia did have fewer vessels for a given shoot length and for a given mean vessel diameter as compared to species with conventional cambia (Fig. 6, Table 3). Carlquist (1975:183) presents a graph of vessel density against vessel diameter, and most species with successive cambia tend to have relatively fewer vessels given their vessel diameter, exactly paralleled by our results (Fig. 6b). The vessels in species with successive cambia remain closer to active phloem for longer periods than in plants with conventional cambia (Fig. 2i). As a result, it seems plausible that plants with successive cambia would be able to regulate conduction and perhaps repair embolisms via loading of photosynthates into the vessels (Robert et al. 2011) over longer periods than species with single cambia, leading to longer vessel functional lifespan. Greater vessel functional lifespan, in turn, would be associated with lower vessel density for a given shoot length, given that longer lived vessels would require less replacement and the

TABLE 5. The effects of imperforate tracheary element type, vasicentric tracheids, and vascular tracheids on vessel diameter- and vessel density- shoot length scaling.

Response variable	N	R ² _{adj}	df	F	Slope equality P	Slope inequality P	Slope	Intercept	Fig.
VD _{base}									
~SL + ITE	639	0.60	5, 633	190.4***	<0.05	<0.001	a, libriform fibers: 0.455 (0.304, 0.606) a,b, fiber tracheids: 0.395(0.324, 0.466) b, tracheids: 0.373(0.212, 0.534) 0.454 (0.422, 0.486)	libriform fibers: 1.455 (1.341, 1.569) fiber tracheids: 1.462 (1.408, 1.517) tracheids: 1.428 (1.309, 1.547)	not shown
Simple perforation plate species: ~SL + ITE	497	0.61	1, 495	777.3***	0.394	0.111	0.419 (0.385, 0.452)	1.452 (1.431, 1.474)	4g
~SL + vasicentric	464	0.62	2, 461	375.9***	0.549	<0.001	0.433 (0.400, 0.466)	absent: 1.490 (1.463, 1.516) present: 1.415 (1.354, 1.476)	4h
~SL + vascular	464	0.61	2, 461	358.8***	0.536	<0.05		absent: 1.468 (1.443, 1.492) present: 1.413 (1.338, 1.488)	4i
VD _{tip}									
~SL + ITE	534	0.24	5, 528	33.73***	<0.05	–	a, libriform fibers: 0.215 (0.061, 0.370) b, fiber tracheids: 0.111 (0.039, 0.184) a, tracheids: 0.193 (0.028, 0.357) 0.198 (0.165, 0.232)	tracheids: 1.044 (0.923, 1.165) fiber tracheids: 1.113 (1.059, 1.168) libriform fibers: 1.055 (0.939, 1.171) 1.056 (1.033, 1.078)	not shown
Simple perforation plate species: ~SL + ITE	406	0.25	1, 404	133.5***	0.094	0.237	0.161 (0.124, 0.198)	absent: 1.111 (1.082, 1.141) present: 1.003 (0.936, 1.071)	4p
~SL + vasicentric	376	0.28	2, 373	73.85***	0.832	<0.001		absent: 1.069 (1.042, 1.096) present: 1.062 (0.960, 1.163)	4q
~SL + vascular	376	0.24	3, 372	40.44***	<0.05	–	absent: 0.199 (0.161, 0.236) present: 0.038 (–0.135, 0.210)		4r
Vmm ² _{base}									
~SL + ITE	613	0.42	3, 609	146.2***	0.058	<0.001	–0.727 (–0.798, –0.656)	a, libriform fibers: 2.147 (2.001, 2.294) a,b, fiber tracheids: 2.224 (2.049, 2.400) b, tracheids: 2.319 (2.248, 2.389) 2.207 (2.152, 2.261)	not shown
Simple perforation plate species: ~SL + ITE	471	0.45	1, 469	386.6***	0.775	0.151	–0.812 (–0.893, –0.731)	absent: 2.107 (2.037, 2.178) present: 2.264 (2.104, 2.424)	5g
~SL + vasicentric	438	0.40	2, 435	167.8***	0.761	<0.001	–0.707 (–0.794, –0.621)	absent: 2.130 (2.068, 2.192) present: 2.407 (2.216, 2.598)	5h
~SL + vascular	438	0.44	2, 435	172.9***	0.864	<0.001	–0.720 (–0.803, –0.636)		5i
Vmm ² _{tip}									
~SL + ITE	478	0.06	1, 476	32.23***	0.279	0.050	–0.171 (–0.231, –0.112)	2.969 (2.930, 3.008)	not shown
Simple perforation plate species: ~SL + ITE	362	0.09	1, 360	34.86***	0.220	0.05	–0.199 (–0.266, –0.133)	2.981 (2.939, 3.024)	5p
~SL + vasicentric	334	0.12	2, 331	23.50***	0.691	<0.001	–0.133 (–0.205, –0.060)	absent: 2.883 (2.827, 2.938) present: 3.054 (2.925, 3.182)	5q
~SL + vascular	334	0.09	2, 331	17.87***	0.125	<0.005	–0.162 (–0.233, –0.091)	absent: 2.926 (2.876, 2.976) present: 3.104 (2.947, 3.261)	5r

Notes: ITE, background imperforate tracheary element; SL, shoot length; VD_{base}, vessel diameter at shoot base; VD_{tip}, vessel diameter at shoot tip; Vmm²_{base}, vessels density at shoot tip. Perforation: scalariform, scalariform perforation plate; simple, simple perforation plate. Successive cambia: yes, presence of successive cambia; no, absence of successive cambia. Notation as for Table 1.

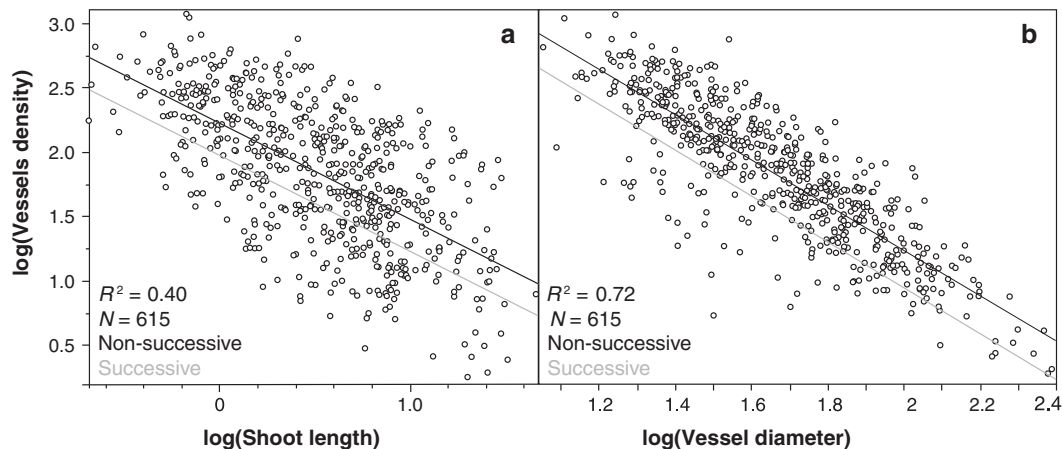


FIG. 6. Vessel density (vessels/mm²) and successive cambia. (a). Species with successive cambia had lower vessel density for a given shoot length (m). (b). Likewise, they also had lower vessel density for a given mean vessel diameter (μm).

concentration of conduction in the outer part of shoots, as occurs in plants with conventional single cambia. Our results thus suggest that the presence of phloem strands innervating the shoot allows for different patterns of trait association to be favored as compared to species with single cambia, to a large degree irrespective of wood density.

Scalariform vs. simple perforation plates.—Our data are congruent with previous observations that species with scalariform perforation plates tend to have narrower vessels than those with simple plates (Carlquist 2001, Medeiros et al. 2018). We found that the VD_{base} –SL slope was significantly lower in species with scalariform perforation plates (Table 3, Fig. 2g), whereas at the shoot tip, simple and scalariform-plated species had

similar VD–SL scaling slopes (Table 3, Fig. 2p). There are very few “dicot” species with wide vessels and scalariform perforation plates, and even these have a much-reduced number of bars on the perforation plates (Bailey and Tupper 1918, Carlquist 1975). Instead, the more common condition is for perforation plates to have numerous (10–40 or even more; Wheeler et al. 2007), slender bars and for vessels to be relatively narrow. Our data shed some light on this pattern. Traditionally, it is said that scalariform-plated species have narrow vessels, but our data show that at moderate shoot lengths, the range of scalariform and simple perforation plate diameters is similar. The novel observation is that the proportions diverge most at longer shoot lengths. This emphasizes that there are very few tall trees, with their correspondingly wide vessels, with scalariform

TABLE 6. Squared loadings for anatomical and functional traits from the principal component analyses for mixed data.

Parameter	PCA 1			PCA 2		
	Dimension 1	Dimension 2	Dimension 3	Dimension 1	Dimension 2	Dimension 3
Density	0.0249	0.5047	0.0137	0.0252	0.0773	0.2899
VD–SL residuals	0.0347	0.5073	0.0615	0.0211	0.0056	0.6674
Successive cambia	0.0149	0.0003	0.0725	–	–	–
Leaf phenology	0.2107	0.1631	0.6613	0.0019	0.7086	0.3931
Porosity type	0.0876	0.1703	0.5180	0.5619	0.4981	0.0703
Perforation plate	0.6926	0.0002	<0.001	–	–	–
Background ITE	0.6178	0.1753	0.016	0.1146	0.0038	0.0513
Vasicentric	–	–	–	0.5351	0.012	0.0763
Vascular	–	–	–	0.4605	0.0214	0.007
Percentage of variance explained	16.8	15.2	13.4	19.1	14.7	17.3
Cumulative percentage of variance explained	16.8	32.0	45.5	19.1	33.8	51.1

Notes: PCA 1 included most species and excluded the presence/absence of vasicentric and vascular tracheids to allow the analyses of all kinds of background imperforate tracheary elements (ITE). Intended to examine patterns of association of vascular and vasicentric tracheids with the other traits, PCA 2 included species without tracheids and excluded the variable of successive cambia and perforation plate type.

Dashes indicate the variables that were not included in a given analysis. Loadings > 0.25 are given in bold

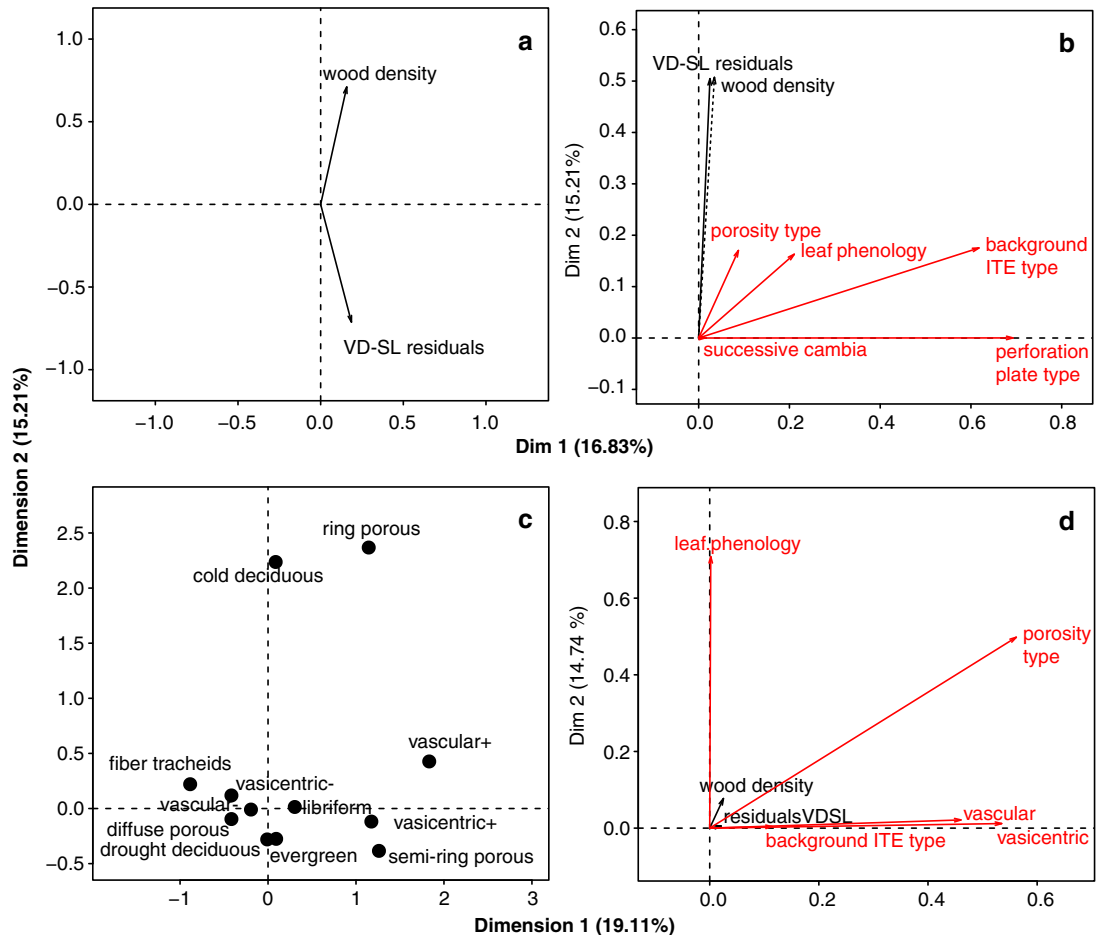


FIG. 7. PCA with continuous and categorical variables, illustrating patterns of association and independence among variables. (a) PCA with the two continuous variables included, which were VD_{base} -SL residuals (i.e., vessel diameter standardized for shoot length), and wood density. The analysis confirms the results Table 6 that, for a given shoot length, plants with lower wood density have wider vessels. (b). Mixed PCA with VD -SL residuals, wood density, and the categorical variables porosity type, perforation plate type, background imperforate tracheary element type, leaf phenology, and successive cambia. This plot shows squared loadings, so they are all in the same (positive) quadrant. We excluded vasicentric and vascular tracheids from this analysis because they don't occur with true tracheids and so would be perfectly negatively correlated. The analysis shows that VD -SL residuals and wood density are negatively correlated, denoted by the dashed line for the wood density arrow. Perforation plate type and imperforate tracheary element type tend to be related, with scalariform-plated species often having true tracheids and simple-plated species often having libriform fibers or fiber tracheids (see text). Species with low wood density tend to be drought deciduous and diffuse porous (see Table 6). The presence or absence of successive cambia is not strongly related to the other variables. (c). PCA plot with only categorical variables, including porosity types, leaf phenology, and vasicentric and vascular tracheid presence (+) or absence (-). It shows that ring porous species are often cold deciduous, that drought deciduous species are often diffuse porous, and that vascular and vasicentric tracheids tend to be found in semi-ring porous species with libriform fibers. (d). Mixed PCA plot of the same variables as in (c), with the addition of the continuous variables VD -SL residuals plus wood density. Together with the previous plot and the loadings in Table 6, it reflects the association between vasicentric and vascular tracheids with libriform fibers and the tendency for these cell types to be found in semi-ring porous species. It also reflects the tendency for drought deciduous species to have lower wood density and therefore slightly wider vessels for a given height.

perforation plates. Globally, scalariform perforation plates only seem to persist (or be favored) in situations of high resistance (Carlquist 2018). These situations include narrow cloud forest vessels, and in some species, latewood and shoot tips. Paralleling patterns in diameter, we also found a correspondingly higher number of vessels per unit of xylem transection in scalariform-plated species, over most size classes, a tendency that was

most pronounced at longer shoot lengths (Table 3, Fig. 3g,p). It has been proposed (Carlquist 1975, 2018) that when high peak conductive rates are favored by selection, wide vessels with simple perforation plates always have higher performance than scalariform ones, because of the higher resistance to flow of scalariform perforation plates (Christman and Sperry 2010). This hypothesis predicts that scalariform perforation plates

TABLE 7. Factors that have been tested for their effect on species mean vessel-diameter–shoot-length scaling.

Variable	Effect	Postulated cause	References
Angiosperms vs. gymnosperms	Shoot base tracheid-diameter–plant-height scaling slope is much lower in conifers than in angiosperms.	Terminal twig tracheid diameter does not widen with shoot height as terminal twig vessel diameter does in angiosperms (Fig 4j).	Liu et al. (2019), Williams et al. (2019)
Earlywood vs. latewood of ring porous species	The earlywood vessels scale similarly to those of diffuse porous species, whereas the latewood vessels scale with a lower slope, that is, are increasingly narrower than expected in taller plants.	Selection favoring narrow, freezing-induced embolism resistance in latewood.	This study
Ground tissue imperforate tracheary element (ITE) type (Table 2)	In species with simple perforation plates, vessel-diameter–stem-length scaling is identical in species with different imperforate tracheary element type.	Libriform fibers, fiber tracheids, and true tracheids do not affect vessel-diameter–plant-height relations, suggesting division of labor between imperforate elements and vessels.	This study
Total leaf area	Variation in total leaf area for individuals of similar heights does not affect the VD–SL relationship. Instead, total vessel number scales with leaf area.	For a given plant height, each unit of leaf area is supplied by a given number of conduits; these conduits widen similarly tip-to-base given conductive path length.	Echeverría et al. (2019)
Leaf phenology	Drought deciduous species have wider vessels for a given height than evergreen species, and cold deciduous species have even narrower vessels for a given height than evergreen species.	Drought deciduous species tend to have lower density wood that is less resistant to embolism, dropping their leaves before highly negative tensions develop. Evergreen and cold deciduous species experience greater tensions, selecting for narrower, more embolism-resistant vessels.	This study
Liana habit	Lianas have wider vessel diameter variances for a given shoot length than self-supporting plants, but their mean vessel diameters scale identically.	With their limited shoot transectional area, selection favors high conductance, hence somewhat wider maximum vessel diameters in lianas, but also the greater safety conferred by more abundant very narrow vessels.	Olson et al. (2014), Rosell and Olson (2014)
Porosity type	Diffuse and ring porous species have wider mean vessel diameters for a given height than semi-ring porous species.	Species with semi-ring porosity tend to be high wood density species in dry or cold areas, where selection favors narrow, embolism-resistant vessels.	This study
Precipitation	No effect; individuals in moist places have on average similar vessel diameters as compared to similar-sized individuals in dry ones.	Selection favors constant conductance per unit leaf area with height growth irrespective of precipitation.	Morris et al. (2017), Olson et al. (2018b)
Simple vs. scalariform perforation plates	Species with scalariform perforation plates scale with a lower y-intercept and a lower slope than species with simple plates (Figs. 4f and 5f).	Scalariform perforation plates are only retained in situations of high resistance (Carlquist 2018), and hence are only seen in the relatively narrowest vessels given plant height.	This study; Medeiros et al. (2018)
Successive cambia	Species with successive cambia tend to have narrower vessels and lower vessel density than those with conventional single cambia.	Vessels in plants with successive cambia remain in proximity to living phloem for a longer time than in species with conventional cambia, and so vessel longevity is longer, allowing for narrower and lower vessel density as compared to species with conventional cambia and vessels with shorter functional lifespans.	This study
Temperature	Species in warmer climates have slightly wider vessels for a given height than those in colder climates.	Selection favoring resistance to freezing-induced embolism cannot explain this pattern, because it is observed even across frost-free communities.	Morris et al. (2017), Olson et al. (2018b)
Vascular tracheids	Species with vascular tracheids tend to have narrower vessels for a given height than those without.	Species with vascular tracheids are mostly dryland species with soft leaves that wither as the dry season progresses; seasonal drought favors narrow, embolism-resistant vessels.	This study
Vasicentric tracheids	Species with vasicentric tracheids tend to have narrower vessels for a given height than those without.	Species with vasicentric tracheids are mostly high wood density, evergreen sclerophyll species of drylands, situations that favor narrow, embolism-resistant vessels.	This study

TABLE 7. Continued.

Variable	Effect	Postulated cause	References
Vestured pits	Species with and without vestures scale with similar slopes, with species with vestures having wider vessels for a given height.	Possibly due to the higher temperatures often associated with species with vesturing (Jansen et al. 2004); see <i>Temperature</i> .	Medeiros et al. (2018)
Wood density	Species with lower wood density have slightly wider vessels for a given height.	Low wood density species tend to be less embolism resistant, and to drop their leaves before highly negative tensions set in. Higher wood density species tend to have longer-lived leaves that persist longer and generate greater tensions, favoring relatively narrower vessels.	This study; Olson et al. (2018b)

should be observed in situations in which high resistance is not selected against. In the samples examined here, when there was within-sample variation in perforation plate type, the scalariform plates were always in situations of high resistance, such as at the shoot tip or in the latewood. Our results showing similar vessel-diameter–shoot-length scaling between scalariform and simple-plated species in the high resistance situation of the shoot tip is congruent with this expectation. Similarly, selection against scalariform plates in situations where low resistance is favored could account, at least in part, for why at larger shoot lengths, species with scalariform perforation plates become increasingly rare, leading to the diverging slopes observed here.

Cold selecting for narrow latewood vessels in ring porous species

The lower scaling slope of latewood vessels in ring porous species is consistent with selection favoring resistance of freezing-induced embolism in these largely cold deciduous species (Fig. 4c). When averaging vessel diameter across growth rings, there was no difference in the scaling slope between ring porous, semi-ring porous, and diffuse porous species (Table 1, Fig. 2b, k). This result is congruent with predictions, such as that of the West, Brown, and Enquist model (West et al. 1999), that large sample sizes should tend to converge in their VD–SL scaling exponent (Olson et al. 2018b). These large sample sizes and means, however, surely mask some biologically meaningful variation. We began to explore this variation here by separating the 12 widest and the 12 narrowest vessels per individuals of the 35 ring porous species included in the data set. These ranged in mean height per species from 0.27 to 28.46 m tall and spanned a wide variety of habitats. All but one of the ring porous species in our data set came from the temperate zone where frosts occur, and most were from moist habitats, so cold rather than drought is the common climatic denominator in these species. The wide earlywood vessels of ring porous plants conduct most water during periods in which freezes are rare and water is abundant (Ellmore and Ewers 1986, Sperry et al. 1994, Kitin and Funada 2016). These usually become non-functional,

and often occluded by tyloses, by the end of the growing season (Cochard and Tyree 1990, Kitin and Funada 2016). In contrast, the narrow latewood vessels remain functional well into the late growing season, as cold sets in (Pérez-de-Lis et al. 2018). Narrower vessels are more resistant to freezing-induced embolism (Pockman and Sperry 1997, Davis et al. 1999, Cavender-Bares and Holbrook 2001, Martínez-Vilalta and Pockman 2002, Pittermann and Sperry 2003, 2006, Cavender-Bares 2005, Sevanto et al. 2012, Medeiros and Pockman 2014). The differences we detected (Table 1, Fig. 2c) suggest that, as ring porous plants get taller, selection favoring disproportionately narrow latewood vessels becomes increasingly pronounced.

While both drought and cold might favor narrower vessels, the data are more consistent with cold rather than drought being the selective agent responsible for the tendency for the VD_{base} –SL slope being lower in latewood than in earlywood in ring porous species (see, for example, Lens et al. 2013, Medeiros and Pockman 2014). If narrow and wide vessels scale with different slopes regardless of their distribution in rings, then even the narrow vessels of diffuse porous species that are not exposed to cold should also scale with a lower slope. To test this possibility, we fit a model describing the VD–SL relationship of the 12 widest and 12 narrowest vessels in the 133 diffuse porous species with wood density in the lowest quartile of our data set (≤ 0.42 g/cm³). These are all species from frost-free, or nearly frost-free localities, and most are fugaciously deciduous, dropping their leaves very early in response to annual drought (Méndez-Alonzo et al. 2012). Given these characteristics, the low wood density species in our data set would least be expected to show the effects of selection of cold conditions in the late growing season. In contrast to the ring porous species, the 12 widest and 12 narrowest vessels in the low wood density species scaled with identical slopes, at the shoot base and tip (Table 3, Fig. 4d, m). Moreover, if cold leads to lower vessel-diameter–shoot-length scaling slopes given increasing costs in terms of mortality downstream in larger individuals, then it should be that cold-deciduous species should have narrower vessels for a given shoot length than evergreen and drought-deciduous species. As we discuss in more

detail below, this was strikingly the case for the VD_{base} –SL relationship, in which cold-deciduous species had significantly narrower vessels for a given shoot height at the shoot base (and a similar VD –SL slope, Table 3, Fig. 4e). At the shoot tip, the slopes were different, with cold deciduous species having a markedly lower VD_{tip} –SL slope than the evergreen or drought deciduous species (Table 3, Fig. 4n), very similar to the pattern observed between early and latewood in ring porous species. Rather than drought, these results are congruent with the notion that the latewood vessels of ring porous species scale with a lower slope than the earlywood because of the differing temperature conditions between the early and late growing season.

Vessel density

There is a long-recognized and well-documented negative relationship between vessel density and vessel diameter across the woody plants (Carlquist 1975, Zanne et al. 2010, Martinez-Vilalta et al. 2012, Rosell and Olson 2014, Pratt and Jacobsen 2017), and this relationship was reflected in all the models we fit here. When a categorical variable was associated with a higher VD –SL intercept, it was dependably associated with a lower Vmm^2 –SL intercept. In biological terms, this means that whenever a factor, such as porosity type or vasicentric tracheid absence, was associated with wider vessel diameters for a given shoot length, there were fewer vessels per unit xylem transection for a given shoot length. The same can be said for cases of slope differences. As the VD –SL slope varied with variables such as those we examined here, e.g., porosity type or leaf phenology, Vmm^2 mirrored this variation (cf. McCulloh et al. 2010). A previous study found that Vmm^2 accounted for 33% of the variation in VD not accounted for by SL (Olson et al. 2014). The significance of this dependable change in VD or Vmm^2 whenever the other is altered is presumably due to selection favoring the maintenance of conduction per unit leaf area despite changes in VD or Vmm^2 (Echeverría et al. 2019).

Tip-to-base changes and functional significance

The VD_{base} –SL and VD_{tip} –SL slopes we observed are consistent with the expectation that vessel diameter should widen from the branch tip toward the base approximating a power law of the form VD –SL^{0.2} or slightly above. Within individuals, the tip-to-base conduit widening profile follows the approximately 0.2 scaling exponent with distance from the tip, throughout the life of the plant as it grows from a sapling to its maximum size (Williams et al. 2019). However, our data and those of other authors (Zach et al. 2010, Olson et al. 2014, 2018b) show that VD_{tip} widens with plant height (Fig. 4, Table 3). Experimental work suggests that this pattern is the result of selection minimizing the increase

in sapwood carbon costs per unit leaf area as plants grow taller (Echeverría et al. 2019). In the only study to date to examine the issue, taller individuals of the tropical tree *Moringa oleifera* had wider VD_{tip} and thus lower per-vessel resistance. Lower per-conduit resistance meant that the same leaf area could be supplied by fewer vessels in a taller individual. This reduction in vessel number per unit leaf area with height growth plausibly contributes to maintaining sapwood carbon costs constant per unit leaf area as plants grow in height and conductive path length increases (Echeverría et al. 2019). VD_{tip} represents the y -intercept of the VD –SL relationship. If VD_{tip} increases as plants get taller, then the y -intercept of the VD_{base} –SL relationship increases. This within-individual ontogenetic pattern must be taken into account in interpreting comparative patterns such as the one studied here. Based on the slopes in Table 3, which show that $VD_{base} \propto SL^{0.430}$, and $VD_{tip} \propto SL^{0.196}$, then the widening rate = $SL^{0.430}/SL^{0.196} = 0.234$, exactly in the predicted range and coinciding with previous studies (West et al. 1999, Petit and Anfodillo 2009, Olson et al. 2014, 2018b, Rosell and Olson 2014, Morris et al. 2018). While a power law is an often adequate approximation, reports of conduit diameter that is slightly narrower than predicted given power law expectations remain to be explained (Petit et al. 2010, Pfautsch et al. 2018, Williams et al. 2019). These considerations notwithstanding, our results, which sampled only at the shoot the tip and base, are nevertheless congruent with the approximately power-law-like form of the within-individual pattern of tip-to-base vessel widening.

With regard to features other than vessel diameter and density, simultaneous examination of large numbers of samples from shoot tips and bases resulted in numerous novel observations that provide a basis for further testing regarding their functional significance. For example, our models testing for differences in VD –SL scaling across phenological categories (drought deciduous, evergreen, and cold deciduous) revealed different intercepts in VD_{base} –SL scaling, with cold-deciduous species having the lowest intercept, as predicted (Fig. 4e). Unexpected, however, was the finding that the VD –SL relationship scaled with a significantly lower slope in cold deciduous species than in the other two at the shoot tip (Fig. 4n). This finding raises the question of whether cold conditions exert selection for relatively narrow vessels in taller plants under cold conditions, and whether the selective conditions at the top of a tree are sufficiently different (e.g., much less thermal mass at the tip) from those at the base to lead to such divergent patterns of trait association as the ones we observed between shoot tip and base. Virtually all these patterns are novel and are congruent with expectations regarding their ecological significance.

Other regularities were observed in the course of microscope observation and require additional study. All these patterns suggest selection favoring xylem

structure with greater embolism resistance at the shoot tip as compared to the base, presumably associated with the gradient in xylem water potential from highly negative at the tip to less negative toward the base (Hellkvist et al. 1974). One such observation was that many species had conspicuously narrow perforations at the shoot tip, but not at the base. Narrow perforations are observed as perforation plates with very wide “collars,” meaning that the perforation is much narrower than the vessel lumen (Fig. 3c). Materials mechanics suggests that the “collars” on the vessels would provide mechanical support in resisting the stress imposed by highly negative xylem potentials within the vessels. Moreover, it could be that even simple perforation plates assist in localizing embolisms (Carlquist 2001) or recovering from them (Brodersen et al. 2018). Whatever the cause, narrow “collars” at the tip was such a common pattern that it would necessarily seem to have functional significance meriting further study. Another such pattern was that many species had apparently conductive imperforate tracheary elements at the shoot tip, but clearly non-conductive elements at the base. This occurred in different modalities. One modality, exemplified by *Clethra alnifolia* (Clethraceae) and *Erica vagans* (Ericaceae), was that the background elements at the tip were all (presumably) conductive tracheids, densely covered with wide-bordered pits, and fiber tracheids or libriform fibers at the shoot base. In other cases, as in *Quercus pyrenaica* (Fagaceae), vasicentric tracheids were very abundant at the shoot tip, and were proportionally much less abundant at the shoot base. Another modality, exemplified by *Arctostaphylos glauca* (Ericaceae), was that vascular tracheids were vastly overrepresented at the tip in comparison with the base. As with narrow perforation plates, all these distributions of imperforate tracheary elements correlate with increased safety against embolism at the shoot tip, again consonant with the greater xylem tensions present there (Tyree and Ewers 1991, Wheeler and Stroock 2008). These observations suggest that, as suggested by previous authors (Jacobsen and Pratt 2018, Rodriguez-Zaccaro et al., 2019, Roddy et al. 2019) that hydraulic function is best thought of as emerging out of the integration of characteristics along the entire conductive path (McCulloh et al. 2019), and that the conspicuous anatomical variation observed along shoots is likely of functional significance (Kocacinar and Sage 2003).

Selection acting on conduit diameter via shoot length and conduit diameter limiting shoot length: correlated evolution in the context of wide developmental potential

Patterns of trait association across the natural world are shaped not only by selection favoring certain variants but also by any biases or gaps in the pool of variants that can be presented to selection (Olson 2012). If a given imaginable trait combination cannot be produced by plant developmental systems, then even if

selection would favor such a combination, it will never be observed (Olson 2019a, b). Xylem that is maximally stiff and provides maximal storage volume would seem to be one such impossible combination, prohibited by the trade-off between stiff wall material and cell lumen (Pratt and Jacobsen 2017). As a result, study of the variants that can be produced (often referred to as “developmental potential” or “evolvability”) is an essential component of efforts to explain patterns of organismal trait association (Donovan et al. 2011, Olson 2012, Olson and Arroyo-Santos 2015). There are certainly important biases in the trait combinations that can be produced in secondary xylem. For example, all axial xylem cells ultimately derive from fusiform cambial initials, and therefore begin development at the same size. This shared developmental origin severely limits the range of size differences that cells such as vessel elements and imperforate tracheary elements can take across species and across ecological contexts (Montes-Cartas et al. 2017). With regard to VD–SL scaling, our results did not provide evidence that this scaling is due to inescapable developmental linkage between shoot dimensions and vessel diameter, but instead because the VD–SL relationship is one favored by selection. This is because the variation about the y-axis is very wide, indicating that a very wide range of vessel diameters can be produced in the context of a given shoot length, illustrating that plant developmental systems are capable of producing vessel diameters much narrower or wider than the mean for a given shoot length. Moreover, hormonal manipulation (Johnson et al. 2018) shows that conduit diameter can be drastically altered for a given plant height, with equally drastic impacts on function, again showing that plants are developmentally capable of producing vastly differing VD–SL trait combinations, but that many of these are likely not favored. The space below the VD–SL scaling line corresponds to vessels that are “too narrow” given shoot length. These variants would have very high resistance and be at a disadvantage as compared to variants with lower resistance and higher photosynthetic rates. The space above the line corresponds to variants with vessels that are “too wide” given shoot length. These individuals would also have lowered productivity or reproductive output given their greater risk of embolism and the higher construction costs per unit leaf area that their wider conduits imply. All of these observations are consistent with the notion that the field of VD–SL developmental possibilities is very wide, and that only certain VD–SL combinations are favored, that is, that VD–SL scaling is an example of correlated evolution, in which many trait combinations are possible but only some are favored. That large samples converge on exactly the VD–SL scaling exponent predicted to maintain conductance constant while minimizing carbon investment as an individual grows taller provides extremely strong evidence for this conclusion, given that no other explanation other than chance seems on offer to explain this

coincidence (West et al. 1999, Petit and Anfodillo 2009, Olson and Arroyo-Santos 2015, Olson et al. 2018a). The VD–SL relationship thus seems clearly an adaptive one, one that, moreover, has slightly differing relationships often favored under differing selective contexts (summarized in Table 7).

The finding that VD scales with SL points to the need to incorporate plant size into thinking regarding many well-known xylem-ecology relationships (Olson et al. 2018b). If SL is the main driver of variation in VD, then selection favoring changes in mean vessel diameter must achieve these changes via changes in plant height. For example, narrower conduits are known to resist freezing-induced embolism better than wide ones (Pockman and Sperry 1997, Davis et al. 1999, Cavender-Bares and Holbrook 2001, Martínez-Vilalta and Pockman 2002, Pittermann and Sperry 2003, 2006, Cavender-Bares et al. 2005, Sevanto et al. 2012, Medeiros and Pockman 2014). More frequent freezing events during the growing season should therefore select for narrow vessels (Zanne et al. 2013), and thus shorter plants. The frequency of freezing relative to the growing season should correlate with maximum vegetation height (Mayr et al. 2002, Mayr and Sperry 2010, Sevanto et al. 2012), and this seems to be the case. Tundra is the shortest vegetation type on Earth, and freezing often occurs during the growing season. Warming of the poles should permit wider conduits and therefore taller plants, consistent with increasing tree and shrub abundance in tundra with climate change (Sturm et al. 2001, Gamache and Payette 2004, Devi et al. 2008, Hallinger et al. 2010, Berner et al. 2013). Similarly, two clones of the same individual tree, one planted in a dry site and the other in a moist one, will grow to differing maximum heights. If wider conduits are more vulnerable to embolisms, and if conduit diameter is determined largely by plant height, then trees should stop growing in height when they reach the maximum mean vessel diameter permitted by the moisture availability that each individual experiences. In this way, the VD–SL relationship is plausibly involved in setting the maximum heights of even genetically identical plants. Our data confirming that height is the main driver of variation in vessel diameter are consistent with height change as the main way that plants respond to selection altering mean conduit diameter. This expectation is consistent with data showing that selection increasing drought resistance via narrower vessels leads to smaller plants (Wyka et al. 2019). Moreover, we also find evidence for small shifts in the VD–SL relationship in different selective contexts. For example, if selection were to favor narrow vessels and vasicentric tracheids in a species of evergreen sclerophyll shrub, it was unknown before that vessel diameter could be narrower than in, say, similar-sized individuals of drought deciduous species with low wood density. This variation is only visible when SL is taken into account.

Other traits potentially affecting scaling: pit membranes, vessel grouping, and vessel length distributions

Models predicting the optimal rate of tip-to-base conduit widening rate are based on very simple assumptions that omit many potentially important biological details that likely influence or covary with VD–SL scaling (West et al. 1999, Petit and Anfodillo 2009, Echeverría et al. 2019). Two of these are vessel length and intervessel pit features. Vessels are made up of vessel elements, which are connected by perforations allowing passage of water. Vessels are of finite length, though, and at each extreme, a vessel is capped with an element that has only one perforation plate. As a result, for water to move from one vessel to the next on its way to the sites of transpiration, it must necessarily pass through the lateral vessel walls. This passage takes place through pit membranes, which are made up of the primary walls of two abutting cells plus the middle lamella. Perforation plates, vessel wall sculpting, and pit membrane characteristics can all presumably affect resistance (Lens et al. 2011, Brodersen et al. 2014, Schenk et al. 2015, Trueba et al. 2019). However, the models used to predict tip-to-base widening rate assume single, continuous, perfect capillary conduits running the lengths of shoots. This very unrealistic model, however, predicts with accuracy the observed widening rate. This prediction can only occur if the features that are omitted from the model, such as vessel length, vessel-vessel pit membrane characteristics, and internal vessel sculpture, scale in perfect proportion with vessel widening from the tip. This expectation seems consistent with the available data. Vessels do appear to become longer tip-to-base, pit membranes become more permeable tip-to-base, and, based on our observations, “collars” and other resistance-imposing internal vessel irregularities become less pronounced tip-to-base, all patterns that seem consistent with the predicted deployment of resistance along shoots (Comstock and Sperry 2000, Sperry et al. 2005, Schulte et al. 2015, Lazzarin et al. 2016). Small changes in these features could potentially affect VD–SL scaling. More resistant pit membranes could be associated with wider vessels (higher VD–SL slopes or intercepts, counteracting the resistance) or more abundant vessels. Similarly, longer vessels could be associated with lower resistance and therefore lower VD–SL intercepts. Another factor is vessel grouping. Martínez-Vilalta et al. (2012) found that species with large vessel groupings had fewer vessels/mm² than did species with solitary vessels. Our results indicate that species with lower vessel densities have higher VD–SL intercepts (Figs. 4, 5). As a result, if grouping affects the vessel-density–SL intercept, it seems likely that it would affect VD–SL scaling as well. Finally, vessel lateral wall pitting is, together with perforation plate type, one of the main descriptors of vessel element morphology. Most species have alternate pitting, though some have opposite or scalariform lateral wall pitting (Frost 1931, Carlquist 2001). Some of the very low wood density

species in our data set had pseudoscalariform lateral wall pitting, which is thought to be derived from alternate pitting in which the pits become very wide in the transverse direction. Because different pitting types can be associated with differing amounts of total pit area per unit vessel lumen, and because pit membrane resistance is an important contributor to total resistance (Sperry et al. 2005), it seems possible that intervessel pit type could influence VD–SL scaling. Species with scalariform lateral wall pitting tend to have longer vessel elements than those with opposite pitting, which are in turn longer than those with alternate pitting (Frost 1931). In turn, scalariform lateral wall pitting tends to be associated with scalariform perforation plates, and alternate pitting with simple plates. As a result of these correlations, distinguishing the effects of lateral wall pitting vs. vessel element length and perforation plate type on VD–SL scaling behavior would likely require large comparative sample sizes. Some traits that are important for whole-plant hydraulic performance likely do not participate in changing VD–SL intercepts or slopes. One of these is leaf area. For a given shoot length within species, vessels appear to widen at the same rate regardless of the total number of vessels present. Total vessel number instead appears to scale with leaf area. Therefore, in two plants of similar height, the individual with greater leaf area will have a thicker stem and a higher total number of vessels, but both will have similar mean vessel diameters at comparable positions throughout their shoots (Echeverría et al. 2019; Rosell and Olson, 2019). There are thus numerous traits whose effects or lack thereof on the VD–SL relationship remain to be tested.

CONCLUSIONS

To date, most inferences of the ecological significance of structural variation and hydraulic performance in woody plants are based on studies that do not take shoot length into account. Yet, because natural selection favors tip-to-base vessel widening as a mechanism that buffers the accumulation of resistance imposed by height growth and longer conductive paths (West et al. 1999, Olson et al. 2018b, Echeverría et al. 2019), it is essential to take plant height into account in such studies. Our approach exemplifies how the functional significance of structural variation can be studied while taking plant height into account. In doing so, we highlight much novel ecological detail. For example, it has been noted for over a century (Bailey and Tupper 1918, Frost 1930) that species with scalariform perforation plates have narrow vessels. We show that this is only the case for larger plants and at the shoot base. Smaller plants with scalariform perforation plates have similar mean vessel diameters as species with simple perforation plates, and at the shoot tips plants of similar heights have similar mean vessel diameters. These unexpected results open the door to further investigation of the ecological significance of these patterns. Other novel outcomes were that ring porous

species have disproportionately narrow vessel diameters in the latewood of large plants, and that species with successive cambia do not have, as was predicted, narrower vessels or lower vessel density as compared to species with conventional single cambia. Species with successive cambia do, however, have fewer vessels per unit xylem cross-sectional area than similar-sized plants with conventional cambia do, consistent with the hypothesis that successive cambia permit greater vessel functional lifespan and thus fewer vessels. This work shows that the variation about the vessel-diameter–shoot-length scaling line is not noise, but biologically meaningful variation likely reflecting the effects of selection favoring slightly different VD–SL relationships in different ecological situations.

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SUPPORTING INFORMATION

Additional supporting information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecm.1410/full>

DATA AVAILABILITY

Data are available from the TRY Plant Trait Database at <https://doi.org/10.17871/TRY.57>